

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No	:	10/814,123	Confirmation No.	:	8039
Applicant	:	Zhang, et al.			
Filed	:	April 1, 2004			
Title	:	Protein Compatible Methods and Compounds for Controlling the Morphology and Shrinkage of Silica Derived from Polyol-Modified Silanes			
TC./A.U.	:	1712			
Examiner	:	Kuo Liang Peng			
Docket No.	:	3244-126 (Formerly 571-932)			

Honorable Commissioner for Patents
P. O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir:

DECLARATION UNDER 37 CFR §1.132

I, John D. Brennan, a citizen of Canada, and resident of Dundas, Ontario, Canada, declare that the following facts are within my knowledge and are true.

1. I reside at 203 Pleasant Avenue, Dundas, Ontario, Canada L9H 3V5.
2. I currently am an Associate Professor in the Department of Chemistry, McMaster University, 1280 Main St. W., Hamilton, Ontario, Canada, L8S 4M1. I also currently hold a Canada Research Chair in Bioanalytical Chemistry.
3. I have been working in the area of bioanalytical chemistry since 1988. My curriculum vitae is attached to this Declaration as Exhibit B.

4. I am an inventor, along with Zheng Zhang, Yang Chen, Jorge Cruz-Aguado, Richard J. Hodgson, Dina Tleugabulova and Michael A. Brook, of the subject matter as claimed in U.S. Patent Application No. 10/814,123 filed April 4, 2004 (hereafter "the Application").

5. I have read and understood the disclosure and claims of the Application.

6. I have read and understood the Office Action that issued on the Application on December 28, 2006. The Examiner is of the view that claims 1-5, 8-10, 38, 40-45 and 47-48 are obvious over Nakanishi688 (US 5,009,688) in view of Gill (J. Am. Chem. Soc., (1998), 120, 8587-8598), claims 1-5, 8-10, 40-45, 47-52, 54-55 and 56 are obvious over Nakanishi875 (US 5,624,875) in view of Gill, claim 38 is obvious over Nakanishi875 in view of Gill and as evidenced by Barkin (US 3,374,103) and claims 53 and 57-61 are obvious over Nakanishi875 in view of Gill. It is my understanding that the Examiner has maintained this objection because the Examiner contends that there is not that much difference in the data showing the results when the hydrolysis and condensation of the silane precursor is performed at pH 11 using either the diglyceryl silane (DGS) of the present invention DGS (Sample 2) vs polyglyceryl silicate (PGS) of Gill (Sample 10) as the precursor.

7. I have read and understood the claims that are attached to this Declaration as Exhibit C that I understand the Applicants are filing in response to the Office Action dated December 28, 2006. My comments below are based on the amended claims in Exhibit C (hereinafter "the amended claims").

8. The Applicants are claiming a biomolecule compatible method that unexpectedly provides bimodal siliceous materials having a meso/macroporous structure that is suitable for chromatographic applications by combining polyol-modified silane precursors (such as DGS) with one or more water soluble polymers under conditions where a phase separation occurs before gelation,

wherein said conditions comprise combining polyol-modified silane precursors with one or more water soluble polymers at a pH in the range of about 4 to 10.

9. We have performed direct side-by-side comparison hydrolysis and condensation reactions of DGS and PGS in the presence of polyethylene oxide (PEO, 10K MW) at pH 10. The reaction conditions, with the exception of pH, are commensurate in scope with those taught in Nakanishi688 or Nakanishi875 in view of Gill. Experimental details, scanning electron microscopy (SEM) images and mercury porosimetry intrusion data of the resulting materials are presented as Exhibit D.

10. The results provided in Exhibit D clearly show that the DGS sample (sample 1) exhibits macroporosity and mesoporosity, where the PGS sample (sample 2) does not. This demonstrates the unexpected results obtained when DGS (a polyol modified silane) is used as the precursor in the preparation of siliceous materials at pH 10, the upper limit of the pH range claimed in the present application. The unexpected results at the lower limit of the pH range were shown in the declaration of Michael A. Brook filed with the Applicants Response dated October 17, 2006.

11. The experimental results provided herewith, combined with those provided in the declaration of Michael A. Brook filed with the Applicants Response dated October 17, 2006, show that DGS, used in the methods claimed by the present Applicants, is fundamentally different from the material(s) prepared in Gill, Nakanishi688 and Nakanishi875. Specifically, in the presence of PEO (10K MW), DGS was the only precursor that provided macroporous material within the pH range claimed in the present application.

12. In summary, I believe that Applicants are entitled to claim a method of preparing bimodal siliceous material by combining polyol-modified silanes with one or more water soluble polymers under conditions where a phase separation

occurs before gelation as specified in the amended claims. I am of the opinion that the amended claims are not obvious in view of Gill in combination with Nakanishi688 or Nakanish875, since the substitution of DGS for the alkoxysilanes used in both of the Nakanishi patents would not be expected to provide the bimodal macro/mesoporous siliceous material that is obtained using the method of the present invention. This is substantiated by the fact that experiments performed in our own labs have demonstrated that PGS, when combined with a water soluble polymer in the method as claimed in the Applicants' application does **not** provide bimodal meso/macroporous siliceous material.

13. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statement and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the Application or patent resulting therefrom.

April 26, 2007
Date



John D. Brennan

Exhibit B

PIN: 50862

John D. Brennan

Section Title

A. NAME

John David Brennan

B. HOME ADDRESS

203 Pleasant Ave.
Dundas, Ontario
L9H 3V5, Canada
Tel: (905) 627-3110

C. BUSINESS ADDRESS

Department of Chemistry
McMaster University
Hamilton, Ontario, L8S 4M1
Tel: (905) 525-9140 Ext. 27033
Fax: (905) 527-9950
e-mail: brennanj@mcmaster.ca
internet: <http://www.chemistry.mcmaster.ca/faculty/brennan/>

D. OTHER PERSONAL DATA

Date of Birth: November 13, 1965
Birthplace: Toronto, Canada
Citizenship: Canadian
Languages: English

E. EDUCATIONAL BACKGROUND

Doctor of Philosophy (Analytical Chemistry)
"Transduction of Selective Reactions by Covalently Immobilized Amphiphile/Enzyme Membranes Containing a Fluorescent Phospholipid" (Ulrich J. Krull, Supervisor)
Department of Chemistry, University of Toronto, 1993

Master of Science (Analytical Chemistry)
"Fluorescence Transduction of an Enzyme-Substrate Reaction by Modulation of the Structure of Lipid Membranes and Surface Stabilized Fatty Acid Membranes for Biosensor Development" (Ulrich J. Krull, Supervisor)
Department of Chemistry, University of Toronto, 1990

Honours Bachelor of Science (Chemistry)
University of Toronto, Department of Chemistry, 1989

F. CURRENT STATUS AT McMASTER

Associate Professor with Tenure, Department of Chemistry, appointed July 1, 2001.

Section Title

G. PROFESSIONAL ORGANIZATIONS

Canadian Society of Chemistry
American Chemical Society
International Sol-Gel Society
Society for Biomolecular Screening
American Society for Mass Spectrometry

H. EMPLOYMENT HISTORY**i) Academic**

Jan 2004 – Visiting Scientist, MDS/Sciex, 77 Four Valley Rd., Concord, ON, L4K 4V8
June 2004 Phone (905) 660-9006

July 2001- Associate Professor, Department of Chemistry, McMaster University,
Present Hamilton, Ontario, L8S 4M1
Phone (905) 525-9140 (ext. 27033)

Sept. 1998- Assistant Professor, Department of Chemistry, McMaster University,
June 2001 Hamilton, Ontario, L8S 4M1
Phone (905) 525-9140 (ext. 27033)

July 1995- Assistant Professor, Department of Chemistry, Brock University,
Aug. 1998 St. Catharines, Ontario, L2S 3A1
Phone (905) 688-5550

ii) Consultations

Feb 2003 – Sept 2005: MDS/Sciex
Jan 2001 – Dec 2002: MDS Proteomics
Sept 2000: BD Biosciences

iii) Other

April 1993- NSERC Postdoctoral Fellow, Department of Chemistry and Biochemistry
June 1995 University of Windsor, Windsor, Ontario, N9B 3P4
Supervisor: Arthur G. Szabo

Sept. 1993- NSERC Postdoctoral Fellow, Institute for Biological Sciences, National Research
March 1994 Council of Canada, Ottawa, Ontario, K1A 0R6
Supervisor: Arthur G. Szabo

1989-1993 Graduate Student and Teaching Assistant in Analytical Chemistry
Erindale College, University of Toronto, Mississauga, Ontario, L5L 1C6
Supervisor: Professor Ulrich J. Krull

Exhibit B

Section Title

PIN: 50862

John D. Brennan

I. SCHOLARLY AND PROFESSIONAL ACTIVITIES

i) Editorial Duties

Guest editor of a special issue of *Analytica Chimica Acta* (Issue 564, March 30, 2006) on Analytical Tools for Proteomics.

Editorial Board member – Journal of Sol-Gel Science and Technology

ii) Grant Selection Committees

DOE Environmental Remediation Science Program Peer Review Committee (2006)

DOE Genomes to Life Peer Review Committee (2003)

NSERC GSC 24/26 ME/MI/MFA Committee (2002)

Chair of NSERC Site visit Committee for an IRC for Jaan Noolandi (May 14, 2002)

iii) Executive Committees

None

iv) Journal Referee

I have acted as a referee for numerous journals, including Nature Materials, Journal of the American Chemical Society, Analytical Chemistry, Journal of Physical Chemistry B, Langmuir, Chemistry of Materials, Physical Chemistry and Chemical Physics, The Analyst, Analytica Chimica Acta, Journal of Materials Chemistry, the Canadian Journal of Biochemistry and Cell Biology, the Canadian Journal of Chemistry and Biophysical Journal. In total, I would estimate that I have reviewed over 250 papers in the last five years, and currently review approximately 1 paper per week.

ii) External Grant Reviews

I have reviewed grants for NSERC, CRC, Alberta Ingenuity Fund, NSF, DOE, Research Corporation, Petroleum Research Fund, Premier's Research Excellence Awards, Steacie Fellowships, the Wellcome Trust and the US Department of Agriculture (SIBR grant). In total I have reviewed ~120 grants.

J. AREAS OF INTEREST

i) Research: Fluorescence spectroscopy, materials chemistry, sol-gel based bioencapsulation, biosensors, high-throughput drug screening, bioaffinity chromatography, mass spectrometry (ESI and MALDI), protein microarrays, protein biophysics and DNA aptamers.

ii) Teaching: Analytical Chemistry, General Physical Chemistry, Photophysics and Fluorescence Spectroscopy, Chemical Sensors, Electrochemistry, Biophysical Methods

iii) Consulting: Consulting in the areas of sol-gel chemistry and high-throughput drug screening.

K. HONOURS

2006 W.A.E. McBryde Medal (For a significant achievement in pure or applied analytical chemistry by a young scientist working in Canada.)

2001-2011 Canada Research Chair in Bioanalytical Chemistry (Teir II).

2001-2011 Ontario Distinguished Researcher.

Exhibit B

Section Title	PIN: 50862	John D. Brennan
1999-2004	Premier's Research Excellence Award, Ontario Ministry of Environment, Science and Technology	
1998	CNC-IUPAC Travel Award	
1993-1995	Natural Sciences and Engineering Research Council of Canada Postdoctoral Fellowship (Two year Award).	
1991-1993	Natural Sciences and Engineering Research Council of Canada Postgraduate Fellowship (PGS3 and PGS4), (Two year Award).	
1990-1991	Natural Sciences and Engineering Research Council of Canada Postgraduate Fellowship (PGS2).	
1989-1990	University of Toronto Open Graduate Fellowship.	
1990	F.E. Beamish Postgraduate Award for best graduate scholar in analytical chemistry.	
1989	Society of Chemical Industry Merit Award for best scholar in final undergraduate year.	
1988	American Chemical Society Award in Undergraduate Analytical Chemistry.	

L. COURSES TAUGHT

i) Undergraduate

A: Brock University, Department of Chemistry

CHEM/BTEC 4P67 Biophysical Techniques 1995-1996, 1997-1998

CHEM/BTEC 2P63 Introduction to Biophysical Chemistry, 1995-1998

CHEM 4P46/5P46 Spectroscopic methods for the analysis of surfaces, 1995-1998

CHEM 3P40 Instrumental Methods of Analysis, 1996-1997

B: McMaster University, Department of Chemistry

CHEM 4P03 Advanced Analytical Chemistry, 2004/2005, 2005/2006, 2006/2007

CHEM 3AO3 Analytical Chemistry II, 2002-2003

CHEM 2PB3 Chemical Thermodynamics and Kinetics, 1998-1999, 2000-2002

CHEM 4TA3 Advanced Analytical Chemistry, 1999-2000

CHEM 2RO3 General Physical Chemistry, 1999-2001

ii) Graduate

A: Brock University, Department of Chemistry

CHEM 5P42 Chemical Sensors, 1996-1997

CHEM 5P10 Photophysics, 1997-1998

B: McMaster University, Department of Chemistry

CHEM 713 Chemical Sensors and Biosensors. 1998-1999, 2000-2001, 2003, 2005

CHEM 710 Electroanalytical Chemistry. 1999-2000, 2001-2002

iii) Postgraduate

None

iv) Other

None

M. SUPERVISORSHIPS

i) Masters

1. Lili Zheng (Complete, January 1996-Dec 1997) "Investigation of Single Tryptophan Proteins Encapsulated in TEOS-Derived Sol-Gel Matrices by Fluorescence Spectroscopy" Brock University.
2. Kulwinder Flora (Complete, September 1996-August 1998) "Fluorimetric Monitoring of the Properties of Proteins Entrapped in Sol-Gel Derived Matrixes." M.Sc. Thesis, Brock University.
3. Mike Rakic (Complete, September 1997-August 1999). "Preparation and Characterization of Organically-Modified Sol-gel Derived Materials: Spectroscopic and Biological Assay Studies for the Development of Optical Biosensors using Sol-Gel Immobilized Proteins and Enzymes." McMaster.
4. Makedonka Gulcev. (Complete, September 2000-August 2003). "The Effects of Doping on the Behaviour of Sol-Gel Entrapped Proteins." McMaster University.
5. Jie Sui (Complete, September 2003-August 2005). "Studies on Electrostatic Interactions Between Biomolecules and Silica Particles Using Time-Resolved Fluorescence Anisotropy". McMaster University
6. Xihua Sui (Complete, September 2003-August 2005). "Characterization and Applications of Sol-Gel Derived Silica Bearing Covalently Tethered Sugars". McMaster University.
7. Katherine Shen (Complete, January 2004 – January 2006). "Secondary Structure Characterization of PH6DZ1, A Fluorescence Signaling and RNA Cleaving DNA Enzyme". McMaster University
8. Jai Sharma (In progress, January 2005 – present)
9. Ivan Partserniak (In progress, May 2005 – present)
10. Roger Luckham (In progress, Sept 2007 – present)

ii) Doctoral

1. Liang Ouyang (In progress, January 2007- present)
2. Gillian Goring (In progress, January 2000- present)
3. Nick Rupcich (Complete, May 2001 – September 2005). "Applications of Biomolecule Entrapment for the Sensing and Screening of Small Molecules".
4. Travis Besanger (Complete, September 2001 – September 2005). "Fundamental Analysis of Membrane-Receptors Entrapped in Sol-Gel Derived Silica: Toward Applications for Drug Screening".

Exhibit B

Section Title

PIN: 50862

John D. Brennan

5. Yujing Gao (Incomplete, May 2002-May 2004).

iii) Postdoctoral

1. Dr. Nicholas Rupcich (October 2005 – July 2006)
2. Dr. Linda Yi (January 2005 – December 2005)
3. Dr. Dora Ilieva (October 2003 – September 2005)
4. Dr. Hanjiang Dong (August 2003 – November 2005)
5. Dr. Jorge Cruz-Aguado (June 2003 – May 2004)
6. Dr. Balu Easwaramoorthy (January 2003 – February 2005)
7. Dr. Richard Hodgson (June 2002 – September 2005)
8. Dr. Dina Thlegubulova (March 2002 – August 2005)
9. Dr. Zhongjie Liu (co-supervised with Dr. Yingfu Li, Dept. of Biochemistry, July 2001- May 2004)
10. Dr. Anil Deisingh (January 2002 – January 2003)
11. Dr. Jianping Xu (co-supervised with Dr. Yingfu Li, Dept. of Biochemistry, March 2002 – March 2003)
12. Dr. Wen Jin (In Progress, January 2001- March 2002)
13. Dr. Hong Long (In Progress, February 2001- March 2002)
14. Dr. Ying Zhang (February 2001- September 2001)
15. Dr. Tracey-Keeling Tucker (September 1999-August 2000)

iv) Professional

Ms. Kulwinder Flora (September 1998 – present) - Technician

Ms. Katherine Shen (February 2006 – present) - Technician

v) Others (Summer Students and Honour's Research Students)

Bill Reid (Jan-Sept 1996)
Sean Musson (May-December 1996)
Brett Wales (May-September 1996)
Bashar Al-Jamal (Jan-April 1997)
Elizabeth Ilnicki (May-Dec 1997)
Cassandra Spong (May-Dec 1997)
Emily DiBattista (Jan-Sept 1998)
Kent Brady (May-August 1996)
Monika Dabrowski (May-August 1996)
Amy Jones (May-August 1996, 1997)
Letting Tu (May-August 1999)
Glenda Bendiak (May-August 1999, 2000)
Doris Yan (May-August 1999, 2000)
Adam Bryant (May-August 2001)
Anna Zavodni (May-August 2001)
Nathan Janzen (May – August 2002, 2003)
Aaron Goldstein (May – August 2002)
Hitesh Bhanabhai (May – August 2002, 2003)
James Green (May – August 2003, 2004, 2005, 2006)
Amy Ticoll (Jan – April 2004)
Domitille Guillon (May – July 2004)
Wai Chin (Sept 2004 – Apr 2005)
Julie Lebert (May – Aug 2006)
Gillian Mackey (May - Aug 2006)
Amit Singhai (May – July 2006)

N. RESEARCH FUNDING (PI is Underlined)

1. Internal Grants

Exhibit B

Section Title

PIN: 50862

John D. Brennan

1. J.D. Brennan (1995). Start-up Funds. Brock University. \$50,000.
2. J.D. Brennan (1996). President's Fund Research Seed Grant. Brock University. \$2,000 Research seed grant. "Fiber Optic Chemical Sensors based on Thin Protein-Doped Silane Films".
3. J.D. Brennan (1998). Start-up Funds. McMaster University. \$100,000.

2. Independent Refereed External Grants Received

1. J.D. Brennan (1996-1999). Individual Research Grant, Natural Science and Engineering Research Council of Canada (NSERC), Research Operating Grant, 29,000/a. "Investigation of protein structure and stability in tetraalkoxysilane derived silica glasses and alkyltriethoxysilane modified glasses using fluorescence techniques.
2. J.D. Brennan (1996). Equipment Grant, NSERC, \$75,341. "High Sensitivity Modular Spectrofluorimeter."
3. J.D. Brennan (1997-1999). Cottrell College Science Awards Program, Research Corporation, Research Grant, \$36,000 (US). "Fiber Optic Chemical Sensors based on Thin Protein-Doped Silane Films."
4. J.D. Brennan (1997). Summer Career Placement Grant, Human Resources and Development Canada, Summer student support grant, \$4,830. "Preparation and Spectroscopic Characterization of Organically Modified Glass Matrices."
5. J.D. Brennan (1998). Travel Award, CNC-IUPAC, Conference Travel Grant \$1,500.
6. J.D. Brennan (1999-2003). Individual Research Grant, NSERC, Research Operating Grant, \$52,500/a. "Development and Characterization of New Methods to Entrap Functional Proteins into Sol-Gel Derived Glasses and Thin Films."
7. J.D. Brennan (1999-2004). Premier's Research Excellence Award, Ministry of Energy, Science and Technology, Ontario, Research Personnel Grant, \$150,000. "Development of a Fluorescence-Based Drug-Screening Assay based on Disruption of Protein-Protein Interactions. (\$100,000 from MEST, \$50,000 from MDS-Sciex).
8. J.D. Brennan (1999). Equipment Grant, NSERC, \$131,694. "Picosecond Fluorescence Lifetime Spectrometer for Characterization of Biomaterials and Biosensor Interfaces."
9. J.D. Brennan (1999). Summer Career Placement Grant, Human Resources and Development Canada, Summer student support grant, \$1,785. "Investigation of a Drug Screening Method using Sol-Gel Entrapped Protein-Protein Complexes."
10. J.D. Brennan (2000). Equipment Grant, NSERC, \$55,037. "Microplate fluorimeter for design of multianalyte sensors and high-throughput drug screening assays."
11. J.D. Brennan (2003-2008). Discovery Grant, NSERC, \$61,300/a. "Fundamental Properties and Analytical Applications of Sol-Gel Derived Biomaterials."

3. Multi-applicant Refereed External Grants Received

4. W.J. Leigh, H. Stover, P. Harrison, F.M. Winnik and J.D. Brennan (1999). Equipment Grant, NSERC, \$98,770. "UV/VIS/NIR Absorption Spectrometer."
5. H. Sheardown, R. Epand, J.L. Brash, R. Pelton and J.D. Brennan (1999). Equipment Grant, NSERC, \$54,141. "Ellipsometer for Characterization of Thin Films."
6. F.M. Winnik, R.F. Childs and J.D. Brennan (1999). Equipment Grant, NSERC, \$132,089. "Scanning and Isothermal Titration Calorimetry System."
7. G. Weatherley, J.D. Brennan and 13 others (1999). Major equipment Grant, NSERC, \$153,114. "Multiple Technique Scanning Probe Microscope."
8. B. McCarry, J.D. Brennan and 16 others (2000). Innovation Fund, Canadian Foundation for Innovation (40%), Ontario Innovation Trust (40%), and private partners (20%), \$12,983,521. "Biomolecular Interactions Initiative".
9. M. Moskovits, G. Wright, E. Brown, J.D. Brennan and 6 others (2000-2005). Ontario Research and Development Challenge Fund, Research Grant within the Ontario Genomics Initiative. "Combinatorial Chemistry and High-Throughput Screening Initiative within the Province of Ontario". \$32,799,000 (1/3 contribution of \$10,933,000 from ORDCF). Approximately \$5.1M of the ORDCF funds will come to McMaster over the 5 year duration of the grant.
10. J.D. Brennan and Y. Li (2001). Defence Research Establishment at Suffield, \$80,800. "Aptamers for sensing of toxin compounds".

Exhibit B

Section Title

PIN: 50862

John D. Brennan

11. J.D. Brennan and M.A. Brook (2004). NSERC RTI-1, \$88,419. "Mercury Intrusion Porosimeter for Characterization of Macroporous Materials".
12. R. Pelton, J.D. Brennan and 18 others (2005-2010). NSERC Research Network Grant, \$2,000,000/a (3% share). SENTINEL: The Canadian Network for the Development and Use of Bioactive Paper".
13. G. Wright, J.D. Brennan and 25 others (2006-2011). Leading Edge Fund, Canadian Foundation for Innovation (40%), Ministry of Research and Innovation, Ontario (40%), and private partners (20%), \$20,070,864. "Center for Microbial Chemical Biology".

4. Industrially Sponsored Grants

1. J.D. Brennan (1999-2000). Research Grant, MDS-Sciex, \$36,900/a. "Development of Sensors and Assays based on Protein-Protein Interactions."
2. J.D. Brennan (2000-2002). Research Grant. MDS-Proteomics, \$78,000/a. "Development of Sensors and Assays based on Protein-Protein Interactions."
3. M. Organ, J.D. Brennan, M. Brook (2000-2003). Collaborative Research and Development Grant, NSERC, Research Operating Grant, \$481,000/a. "Accelerating Drug Discovery Using Frontal Affinity Chromatography/Mass Spectrometry." (\$270,000/a for McMaster). (30% share)
4. J.D. Brennan and Y. Li (2001-2003). MDS-Sciex, \$232,000. "Aptamers for developing biosensors". (50% share)
5. J.D. Brennan (2001). MDS-Sciex, \$12,000. "Development of Assays to Disrupt Protein-Protein Interactions"
6. J.D. Brennan, M.A. Brook, D. Pinto and D. Volmer (2003-2007). NSERC/NRC Research Partnerships Agreement Grant, \$1,127,000 total. "Development of Mesoporous Monolithic Chromatography Columns for High-Throughput Proteomics Applications" (40% share)
7. Y. Li, J.D. Brennan and E.D. Brown (2003-2006). NSERC CRD Grant, \$1,100,000 total. "Signaling Aptamer Microarrays for Surrogate Ligand-Based High Throughput Screening". (30% share)

O. LIFETIME PUBLICATIONS (PI is Underlined)

i) Peer Reviewed

a) Books

None

b) Contributions to Books

1. J.D. Brennan, D.P. Nikolelis and U.J. Krull. Physical Processes of Signal Transduction by Lipid Membranes: Analytical Applications in Biosensor Technology. In: P.T. Frangopol (Ed.) Modern Biophysics: Problems Vol. 2, 1993, pp. 140-199.
2. J.D. Brennan, R.S. Brown, R.K. Kallury, V. Ghaemmaghami M. Thompson and U.J. Krull. Immobilization of Amphiphilic Membranes for Development of Optical and Electrochemical Biosensors. Chemically Modified Surfaces (Proceedings of the 4th Symposium on Chemically Modified Surfaces), D.A. Mottola and J.R. Steinmetz (Eds.), (1992) 275-305.
3. J.D. Brennan, R.F. De Bono, K.M.R. Kallury and U.J. Krull. Ellipsometry, X-ray Photoelectron Spectroscopy and Surface Plasmon Resonance as Techniques for the Study of Chemically Modified Surfaces. Chemically Modified Surfaces (Proceeding of the Fifth Symposium on Chemically Modified Surfaces), J.J. Pesek and L.E. Leigh, (Eds.), 1994, pp. 72-90.
4. R.S. Brown and J.D. Brennan. Optical Spectroscopy of Langmuir-Blodgett and Related Membrane Systems. In: U.J. Krull and D.P. Nikolelis (Eds.) Current Topics in Biophysics, Volume 4, 1995, pp. 32-56.
5. P.A.E. Piunno, J.D. Brennan and U.J. Krull. Fluorescent Chemically-selective Lipid Membrane Sensors using Fiber Optic Systems. In: U.J. Krull and D.P. Nikolelis (Eds.) Current Topics in Biophysics, Volume 4, 1995, pp. 58-86.

Exhibit B

Section Title

PIN: 50862

John D. Brennan

6. D. Tleugabulova and J.D. Brennan*. Time-Resolved Fluorescence Anisotropy Applied to Silica Sol-Gel Growth and Surface Modification. In: *Reviews in Fluorescence 2006*, C.R. Geddes and J.R. Lakowicz (Eds.) Springer, New York, **2006**, pp. 277-309. **(Invited Review Chapter)**
7. N. Rupcich and J. D. Brennan*. Fabrication of Sol-Gel Derived Protein Microarrays for Diagnostics and Screening. In: *Functional Protein Microarrays: From Pathways to Drug Discovery*, Paul Predki (Ed.), CRC Press, **2007**, accepted April 12, 2006. **(Invited Review Chapter)**

c) Journal Articles (* denotes corresponding author, % contribution to joint papers in noted)

96. Y. Yi, D.Y. Chen, M.A. Brook and J.D. Brennan*. Development of Macroporous Titania Monoliths by a Biocompatible Method. Part 2: Enzyme Entrapment Studies. *Chemistry of Materials* **2006**, *18*, 5336-5342.
95. D.Y. Chen, Y. Yi, J.D. Brennan and M.A. Brook*. Development of Macroporous Titania Monoliths by a Biocompatible Method. Part 1: Material Fabrication and Characterization. *Chemistry of Materials* **2006**, *18*, 5326-5335.
94. H. Dong and J.D. Brennan*. Macroporous Monolithic Methylsilsesquioxanes Prepared by a Two-Step Acid/Acid Processing Method. *Chemistry of Materials*, **2006**, *18*, 4176-4182.
93. Y. Shen, W. Chiuman, J.D. Brennan* and Y. Li*. Catalytic and Signaling Properties of Trans-Acting pH6DZ1, an RNA-Cleaving and Fluorescence-Signaling Deoxyribozyme with a Four-Way Junction Structure. *ChemBioChem* **2006**, *7*, 1343-1348. (50%)
92. T.R. Besanger* and J.D. Brennan*. Entrapment of Membrane Proteins in Sol-Gel Derived Silica. *Journal of Sol-Gel Science and Technology* **2006**, *40*, 209-225.
91. J. Sharma, D. Tleugabulova W. Czardybon and J.D. Brennan*. Two-Site Ionic Labeling with Pyranine: Implications for Structural Dynamics Studies of Free and Entrapped Polymers and Polypeptides by Time-Resolved Fluorescence Anisotropy. *Journal of the American Chemical Society*, **2006**, *128*, 5496-5505.
90. N. Rupcich, R. Nutiu, Y. Li* and J.D. Brennan*. Solid-Phase Enzyme Activity Assay Utilizing an Entrapped Fluorescence-Signaling DNA Aptamer. *Angewandte Chemie, International Edition in English*, **2006**, *45*, 3295-3299. (50%)
89. T.R. Besanger, R.J. Hodgson, J.R.A. Green and J.D. Brennan*. Immobilized Enzyme Reactor Chromatography: Optimization of Protein Retention and Enzyme Activity in Monolithic Silica Stationary Phases. *Analytica Chimica Acta* **2006**, *564*, 106-115.
88. T.R. Besanger, R.J. Hodgson, D. Guillon and J.D. Brennan*. Monolithic Membrane-Receptor Columns: Optimization of Column Materials for Frontal Affinity Chromatography/Mass Spectrometry Applications. *Analytica Chimica Acta* **2006**, *561*, 107-118.
87. X. Sui, T.-Y. Lin, D. Tleugabulova, Y. Chen, M.A. Brook and J.D. Brennan*. Monitoring the Distribution of Covalently Tethered Sugar Moieties in Sol-Gel Based Silica Monoliths with Fluorescence Anisotropy: Implications for Entrapped Enzyme Activity. *Chemistry of Materials* **2006**, *18*, 887-896. (80%)
86. D. Tleugabulova and J.D. Brennan*. Quantifying Surface Coverage of Colloidal Silica by a Cationic Peptide using a Combined Centrifugation/Time-Resolved Fluorescence Anisotropy Approach. *Langmuir*, **2006**, *22*, 1852-1857.
85. H. Dong and J.D. Brennan*. Controlling the Morphology of Methylsilsesquioxane Monoliths Using a Two-Step Processing Method. *Chemistry of Materials* **2006**, *18*, 541-546.
84. Nicholas Rupcich, William Chiuman, Razvan Nutiu, Shirley Mei, Kulwinder K. Flora, Yingfu Li* and John D. Brennan*. Quenching of Fluorophore-labeled DNA Oligonucleotides by Divalent Metal Ions: Implications for Selection, Design and Applications of Signaling Aptamers and Signaling Deoxyribozymes. *Journal of the American Chemical Society* **2006**, *128*, 780-790. (50%)
83. H. Dong, R. Riedy and J.D. Brennan*. Shrinkage and Springback Behaviour of Methylsilsesquioxanes Prepared by an Acid/Base Two-Step Processing Procedure. *Chemistry of Materials*, **2005**, *17*, 6012-6017.
82. N. Rupcich, J.R.A. Green and J.D. Brennan*. Nanovolume Kinase Inhibition Assay Using a Sol-Gel Derived Multi-Component Microarray. *Analytical Chemistry* **2005**, *77*, 8013-8019.
81. R. Hodgson, T.R. Besanger, M.A. Brook and J.D. Brennan*. Inhibitor Screening using Immobilized Enzyme-Reactor Chromatography/Mass Spectrometry. *Analytical Chemistry*, **2005**, *77*, 7512-7519. (80%)

Exhibit B

- Section Title PIN: 50862 John D. Brennan
80. Y. Shen, J.D. Brennan and Y. Li*. Characterization of a Fluorescence-Signaling and RNA-Cleaving Deoxyribozyme. *Nucleic Acids Symposium Series*, **2005**, 49, 55-56. (25%)
 79. Y. Shen, J.D. Brennan and Y. Li*. Characterizing the Secondary Structure and Identifying Functionally Essential Nucleotides of pH6DZ1, A Fluorescence-Signaling and RNA-Cleaving Deoxyribozyme. *Biochemistry* **2005**, 44, 12066-12076. (25%)
 78. J.D. Brennan. Biocomposites: Using Light to Drive Biosynthesis. *Nature Materials* **2005**, 4, 189-190.
 77. X. Sui, J. A. Cruz-Aguado, D.Y Chen, Z. Zhang, M.A. Brook and J.D. Brennan*. Effects of Covalently Tethered Sugars on the Properties of Human Serum Albumin Entrapped in Sol-Gel Derived Silica. *Chemistry of Materials*, **2005**, 17, 1174-1182. (80%)
 76. T. Besanger, H. Bhanabhai and J.D. Brennan*. Interferences in Fluo-3 Based Ion-Flux Assays for Ligand Gated Ion Channels. *Analytica Chimica Acta*, **2005**, 537, 125-134.
 75. D. Tleugabulova, J. Siu, P. Ayers and J.D. Brennan*. Evidence for Rigid Binding of Rhodamine 6G to Silica Surfaces in Aqueous Solution based on Fluorescence Anisotropy Decay Analysis. *Journal of Physical Chemistry B*, **2005**, 109, 7850-7858. (90%)
 74. J. Sui, D. Tleugabulova and J.D. Brennan*. Direct and Indirect Monitoring of Peptide-Silica Interactions using Time-Resolved Fluorescence Anisotropy. *Langmuir*, **2005**, 21, 4996-5001.
 73. P. Kovarik, R.J. Hodgson, T.R. Covey, M.A. Brook and J.D. Brennan*. Capillary Scale Frontal Affinity Chromatography/MALDI Tandem Mass Spectrometry using Protein Doped Monolithic Silica Columns. *Analytical Chemistry*, **2005**, 77, 3340-3350. (80%)
 72. H. Dong, M.A. Brook and J.D. Brennan*. A New Route to Monolithic Methylsilsesquioxanes: Gelation Behavior of Methyltrimethoxysilane and Morphology of Resulting Methylsilsesquioxanes Under One-Step and Two-Step Processing. *Chemistry of Materials*, **2005**, 17, 2807-2816. (80%)
 71. Z. Zheng, Y. Chen, R.J. Hodgson, M.A. Brook* and J.D. Brennan. Macroporous Silica Monoliths Derived from Glyceroxysilanes: Controlling Gel Formation and Pore Structure. *Macromolecular Symposia*, **2005**, 226, 253-261. (20%)
 70. N. Rupcich, R. Nutiu, Y. Li and J.D. Brennan*. Entrapment of Fluorescent Signaling DNA Aptamers in Sol-Gel Derived Silica. *Analytical Chemistry*, **2005**, 77, 4300 - 4307. (80%)
 69. R.J. Hodgson, Michael A. Brook and J.D. Brennan*. Capillary-Scale Monolithic Immunoaffinity Columns for Immunoextraction with In-Line Laser Induced Fluorescence Detection. *Analytical Chemistry*, **2005**, 77, 4404 - 4412. (80%)
 68. Y. Chen, Z. Zhang, X. Sui, J.D. Brennan and M.A. Brook*. Reduced Shrinkage of Silica Sol-Gels Using Sugar-based Silsesquioxane Precursors. *Journal of Materials Chemistry*, **2005**, 15, 3132-3141. (20%)
 67. T. Besanger, B. Easwaramoorthy and J.D. Brennan*. Entrapment of Highly Active Membrane-Bound Receptors in Macroporous Sol-Gel Derived Materials. *Analytical Chemistry*, **2004**, 76, 6470-6475.
 66. M.A. Brook,* Y. Chen, K. Guo, Z. Zhang, W. Jin, A. Deisingh and J.D. Brennan*. Proteins Entrapped in Silica Monoliths Prepared from Glyceroxysilanes. *Journal of Sol-Gel Science and Technology*, **2004**, 31, 343-348. (50%)
 65. D. Tleugabulova and J.D. Brennan*. Using Time-Resolved Fluorescence Anisotropy to Assess Side-Chain and Segmental Motions in Polyamines Entrapped in Sol-Gel Derived Silica. *J. Phys. Chem. B*, **2004**, 10692-10699.
 64. K.A. Stephenson, S.R. Banerjee, T.R. Besanger, O. Sogbein, M.K. Levadala, N. McFarlane, J.A. Lemon, D.R. Boreham, K.P. Maresca, J.D. Brennan, J.W. Babich, J. Zubieta* and J.F. Valliant*. Bridging the Gap Between In Vitro and In Vivo Imaging. Iso-Structural Re and ^{99m}Tc Complexes for Correlating Fluorescence and Radioimaging Studies. *J. Am. Chem. Soc.*, **2004**, 8598-8599 (10%).
 63. D. Tleugabulova, A.M. Duft, Z. Zhang, Y. Chen, M.A. Brook and J.D. Brennan*. Evaluating Growth Mechanisms of Silica Particles using Fluorescence Anisotropy Decay Analysis. *Langmuir*, **2004**, 5924-5932. (M)
 62. J. Cruz-Aguado, Y. Chen, M.A. Brook and J.D. Brennan*. Activity and Inhibition of Src Protein Tyrosine Kinase Entrapped within Sugar-Modified Sol-Gel Derived Silica. *Anal. Chem.* **2004**, 76, 4182-4188.
 61. J. Cruz-Aguado, Y. Chen, Z. Zhang, N. Elowe, M.A. Brook and J.D. Brennan*. Ultrasensitive ATP Detection Using Firefly Luciferase Entrapped in Sugar-Modified Sol-Gel Derived Silica. *J. Am. Chem. Soc.* **2004**, 126, 6878-6879. (80%)

Exhibit B

- Section Title PIN: 50862 John D. Brennan
60. R. Hodgson, Y. Chen, Z. Zhang, D. Tleugabulova, H. Long, X. Zhao, M. G. Organ, M. A. Brook and J. D. Brennan*. Protein-Doped Monolithic Silica Columns for Capillary Liquid Chromatography Prepared by the Sol-Gel Method: Applications to Frontal Affinity Chromatography. *Anal. Chem.* **2004**, *76*, 2780-2790. (70%)
59. M.A. Brook,* Y. Chen, **K. Guo**, Z. Zhang and J.D. Brennan. Sugar-Modified Silanes: Precursors for Silica Monoliths. *J. Mater. Chem.* **2004**, *14*, 1469-1479. (20%)
58. D. Tleugabulova, Z. Zhang, Y. Chen, M.A. Brook and J.D. Brennan*. Fluorescence Anisotropy in Studies of Solute Interactions with Covalently Modified Colloidal Silica Nanoparticles. *Langmuir*, **2004**, *20*, 848-854. (80%)
57. D. Tleugabulova, A. M. Duft, M.A. Brook and J.D. Brennan*. Monitoring Solute Interactions with PEO Modified Colloidal Silica Nanoparticles via Fluorescence Anisotropy Decay. *Langmuir*, **2004**, *20*, 101-108. (80%)
56. N. Rupcich and J.D. Brennan*. Coupled Enzyme Reaction Microarrays based on Pin-Printing of Sol-Gel Biomaterials. *Analytica Chimica Acta*, **2003**, *500*, 3-12.
55. T.R. Besanger, Yang Chen, Anil K. Deisingh, Richard Hodgson, Wen Jin, Stanislas Mayer, Michael A. Brook and John D. Brennan*. Screening of Inhibitors using Enzymes Entrapped in Sol-Gel Derived Materials. *Analytical Chemistry*, **2003**, *75*, 2382-2391. (80%)
54. Z. Liu, S.H.J. Mei, J.D. Brennan and Y. Li*. An Assemblage of Signaling DNA Enzymes with Intriguing Metal-Ion Specificities and pH Dependences. *Journal of the American Chemical Society*, **2003**, *125*, 7539-7545. (25%)
53. D. Tleugabulova, Z. Zhang and J.D. Brennan*. Effect of Glycerol on the Evolution of Sodium Silicate Sols Through the Sol-to-Gel Transition. *Journal of Physical Chemistry B*, **2003**, *107*, 10127-10133.
52. S. Mei, Z. Liu, J.D. Brennan and Y. Li*. An Efficient RNA-cleaving DNA Enzyme That Synchronizes Catalysis with Fluorescence Signalling. *Journal of the American Chemical Society*, **2003**, *125*, 412-420. (25%)
51. J.D. Brennan*, D. Benjamin and E. Dibattista and Makedonka D. Gulcev. Using Sugar and Amino Acid Additives to Stabilize Enzymes Within Sol-Gel Derived Silica. *Chemistry of Materials*, **2003**, *15*, 737-745.
50. T. Besanger and J.D. Brennan*. Ion Sensing and Inhibition Studies using the Transmembrane Ion-Channel Peptide Gramicidin A Entrapped in Sol-Gel Derived Silica. *Analytical Chemistry*, **2003**, *75*, 1094-1101.
49. N. Rupcich, A. Goldstein and J.D. Brennan*. Optimization of Sol-Gel Formulations and Surface Treatment for the Development of Pin-Printed Protein Microarrays. *Chemistry of Materials*, **2003**, *15*, 1803-1811.
48. D. Gulcev, G. Goring, M. Rakic and J.D. Brennan*. Reagentless pH-Based Biosensing using a Fluorescently-Labelled Dextran Co-entrapped with a Hydrolytic Enzyme in Sol-Gel Derived Nanocomposite Films. *Analytica Chimica Acta*, **2002**, *457*, 47-59.
47. W. Jin and J.D. Brennan*. Properties and Applications of Proteins Encapsulated Within Sol-Gel Derived Materials. *Analytica Chimica Acta*, **2002**, *461*, 1-36. **(Invited Review)**.
46. T. Besanger, Y. Zhang and J.D. Brennan*. Characterization of Fluorescent Phospholipid Vesicles Entrapped in Sol-Gel Derived Silica. *Journal of Physical Chemistry*, **2002**, *106*, 10535-10542.
45. K.K. Flora, T. Keeling-Tucker, C.W.V. Hogue and J.D. Brennan*. Screening of Antagonists Based on Induced Dissociation of a Calmodulin-Melittin Interaction Entrapped in a Sol-Gel Derived Matrix. *Analytica Chimica Acta*, **2002**, *470*, 19-28.
44. G. Goring, and J.D. Brennan*. Fluorescence and Physical Characterization Sol-Gel Derived Nanocomposite Films Suitable for the Entrapment of Biomolecules. *Journal of Materials Chemistry*, **2002**, *12*, 3400-3406.
43. D. Tleugabulova, Z. Zhang and J.D. Brennan*. Characterization of Bodipy Dimers Formed in a Molecularly Confined Environment. *Journal of Physical Chemistry B*, **2002**, *106*, 13133-13138.
42. J.D. Brennan*, A. Capretta, K. Yong, D. Gerittsma, K.K. Flora, and A. Jones. Sensitization of Lanthanides by Non-Natural Amino Acids. *Photochemistry and Photobiology*, **2002**, *75*, 117-121. (50%)
41. K.K. Flora and J.D. Brennan*. Effect of Matrix Aging on the Behaviour of Human Serum Albumin Entrapped in a Tetraethylorthosilicate-Derived Glass. *Chemistry of Materials*, **2001**, *13*, 4170-4179.

Exhibit B

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|---------------|------------|-----------------|
| Section Title | PIN: 50862 | John D. Brennan |
|---------------|------------|-----------------|
40. K.K. Flora and J.D. Brennan*. Characterization of the Microenvironments of PRODAN Entrapped in an Aging Tetraethylorthosilicate Derived Glass. *Journal of Physical Chemistry*, **2001**, *105*, 12003-12010.
 39. T. Keeling-Tucker and J.D. Brennan*. Fluorescence Probes as Reporters on the Local Structure and Dynamics in Sol-Gel Derived Nanocomposite Materials. *Chemistry of Materials*, **2001**, *13*, 3331-3350. **(Invited Review)**.
 38. T. Keeling-Tucker, M. Rakic, C. Spong and J.D. Brennan*. Control of the Material Properties and Biological Activity of Lipase within Sol-Gel Derived Bioglasses via Organosilane and Polymer Doping. *Chemistry of Materials*, **2000**, *12*, 3695-3704.
 37. J.D. Brennan*. Fluorescence-based Biosensors. *Encyclopedia of Analytical Chemistry*, **2000**, 383-403. **(Invited Review Article)**
 36. J.D. Brennan*, K.K. Flora, G. Bendiak, G.A. Baker M. Kane, S. Pandey and F.V. Bright. Probing the Origins of Spectroscopic Responses to Analyte-Induced Conformational Changes in a Fluorescently-labeled Regulatory Protein. *Journal of Physical Chemistry B*, **2000**, *104*, 10100-10110. (80%)
 35. J.D. Brennan*. Preparation and Entrapment of Fluorescently-labeled Proteins for the Development of Reagentless Optical Biosensors. *Journal of Fluorescence*, **1999**, *9*, 295-312. **(Invited review article)**.
 34. K. Flora and J.D. Brennan*. Comparison of Formats for the Development of Fiber-Optic Biosensors Utilizing Sol-Gel Derived Materials Entrapping Fluorescently-Labelled Proteins. *Analyst*, **1999**, *124*, 1455-1462.
 33. J.D. Brennan*, M.A. Dabrowski, K.K. Flora and S.P. Musson. The Effect of Preparation and Aging Conditions on the Internal Environment of Sol-Gel Derived Matrices as Probed by 7-Azaindole and Pyranine Fluorescence. *Canadian Journal of Chemistry*, **1999**, *77*, 1617-1625.
 32. J.D. Brennan*, J.S. Hartman, E.I. Ilnicki and M. Rakic. Fluorescence and NMR Characterization and Biomolecule Entrapment Studies of Sol-Gel Derived Organic-Inorganic Composite Biomaterials Formed by Sonication of Precursors. *Chemistry of Materials*, **1999**, *11*, 1853-1864. (80%)
 31. J.D. Brennan*. Using Intrinsic Fluorescence to Investigate Proteins Entrapped in Sol-Gel Derived Materials. *Applied Spectroscopy*, **1999**, *53*, 106A-121A. **(Invited Focal Point Article)**
 30. L. Zheng, K.K. Flora and J.D. Brennan*. Improving the Performance of a Sol-Gel Entrapped Metal-Binding Protein by Maximizing Protein Thermal Stability Before Entrapment. *Chemistry of Materials*, **1998**, *10*, 3974-3983.
 29. K. Flora and J.D. Brennan*. Fluorimetric Sensing of Ca²⁺ Based on an Induced Change in the Conformation of Sol-Gel Entrapped Parvalbumin. *Analytical Chemistry*, **1998**, *70*, 4505-4513.
 28. L. Zheng and J.D. Brennan*. Measurement of Intrinsic Fluorescence to Probe the Conformational Flexibility and Thermodynamic Stability of a Single Tryptophan Protein Entrapped in a Sol-Gel Derived Glass Matrix. *Analyst*, **1998**, *123*, 1735-1744.
 27. K. Flora, J.D. Brennan*, G.A. Baker, M.A. Doody and F.V. Bright. Unfolding of Acrylodan Labeled Human Serum Albumin Probed by Steady-State and Time-Resolved Fluorescence Methods. *Biophysical Journal*, **1998**, *75*, 1084-1096. (80%)
 26. L. Zheng, C.W.V. Hogue and J.D. Brennan*. Effects of Metal Binding Affinity and Metal Loading on the Chemical and Thermal Stability of Site-directed Mutants of Rat Oncomodulin. *Biophysical Chemistry*, **1998**, *71*, 157-172.
 25. J.D. Brennan, R.S. Brown and U.J. Krull*. Transduction of Analytical Signals by Supramolecular Assemblies of Amphiphiles Containing Heterogeneously Distributed Fluorophores. *Analytical Sciences*, **1998**, *14*, 141-149.
 24. L. Zheng, W.R. Reid and J.D. Brennan*. Measurement of fluorescence from tryptophan to probe the environment and reaction kinetics within protein-doped sol-gel derived glass monoliths. *Analytical Chemistry*, **1997**, *69*, 3940-3949.
 23. J.D. Brennan, C.W.V. Hogue, B. Rajendran, K.J. Willis and A.G. Szabo*. Preparation of enantiomerically pure L-7-azatryptophan by an enzymatic method and its application to the development of a fluorimetric activity assay for tryptophanyl-tRNA synthetase. *Analytical Biochemistry*, **1997**, *252*, 260-270.
 22. J.D. Brennan, R.S. Brown, H. Cohen, J. Egamino, D. Semchyschyn, R.E. Williams and U.J. Krull*. Towards a homogeneous fluorescence assay for a herbicide: Characterization of the interactions of

Exhibit B

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|---------------|------------|-----------------|
| Section Title | PIN: 50862 | John D. Brennan |
|---------------|------------|-----------------|
- fusilade, bromo-methylmethoxy coumarin derivatized fusilade and anti-fusilade IgG antibody. *Analytica Chimica Acta*, **1996**, 336, 157-166.
21. U.J. Krull*, M.S. Heimlich, K.M.R. Kallury, P.A.E. Piuino, J.D. Brennan, R.S. Brown and D.P. Nikolelis. Investigations of Organized Monolayer Films for Biosensor Development. *Canadian Journal of Chemistry*, **1995**, 73, 1239-1250.
 20. J.S. Shrive, J.D. Brennan, R.S. Brown and U.J. Krull*. Optimization of the Self-Quenching Response of Nitrobenzoxadiazole Dipalmitoylphosphatidylethanolamine in Phospholipid Membranes for Biosensor Development. *Applied Spectroscopy*, **1995**, 49, 304-313.
 19. A. Wang, J.D. Brennan, A. Gene and U.J. Krull*. Assembly of Antibodies in Lipid Membranes for Biosensor Development. *Applied Biochemistry and Biotechnology*, **1995**, 53, 163-181.
 18. K.M.R. Kallury, J.D. Brennan and U.J. Krull*. Depth Profiling of Functionalized Silane Films on Quartz and Silicon Substrates and of Urease Immobilized on Such Films by Angle-Resolved X-ray Photoelectron Spectroscopy. *Analytical Chemistry*, **1995**, 67, 2625-2634.
 17. J.D. Brennan, I.D. Clark, C.W.V. Hogue, A. Ito, L. Juliano, A. Paiva, B. Rajendran and A.G. Szabo*. Interaction Of Enantiomers Of Lysyl-7-Azatriptophyl-Lysine With Acidic Phospholipid Vesicles: A Fluorescence Study. *Applied Spectroscopy*, **1995**, 49, 51-59.
 16. R.S. Brown, J.D. Brennan and U.J. Krull*. Self-Quenching of Nitrobenzoxadiazole Phosphatidylethanolamine in Lipid Membranes. *Journal of Chemical Physics*, **1994**, 100, 6019-6027.
 15. J.D. Brennan, K.M.R. Kallury and U.J. Krull*. Transduction of the Reaction between Urea and Covalently Immobilized Urease by Fluorescent Amphiphilic Membranes. *Thin Solid Films*, **1994**, 244, 898-904.
 14. U.J. Krull*, S.L. Seethaler, J.D. Brennan and D.P. Nikolelis. Bilayer Lipid Membranes as Electrochemical Switches in Reactions Involving Alterations of Surface Charge. *Thin Solid Films*, **1994**, 244, 917-922.
 13. J.D. Brennan, V. Kukavica, K.M.R. Kallury and U.J. Krull*. Fluorimetric Measurement of the Activity of Surface Immobilized Monolayers of Enzyme using a Dual Emission Probe. *Canadian Journal of Chemistry*, **1994**, 72, 721-728.
 12. R.S. Brown, J.D. Brennan and U.J. Krull*. An Optical Fiber Based Spectrometer for Measurement of Fluorescence Lifetimes. *Microchemical Journal*, **1994**, 50, 337-350.
 11. J.D. Brennan, R.S. Brown, A.D. Della Manna, R.K. Kallury, P. Piuino and U.J. Krull*. Covalent Immobilization of Amphiphilic Membranes Containing Urease onto Optical Fibers for Fluorimetric Detection of Urea. *Sensors and Actuators B: Chemical*, **1993**, 11, 109-119.
 10. D.P. Nikolelis, J.D. Brennan, R.S. Brown and U.J. Krull*. Control of Ion Transport Across Bilayer Lipid Membranes by Adjustment of Surface Charge Associated with Phase Domain Structures. *Analytica Chimica Acta*, **1992**, 257, 49-57.
 9. J.D. Brennan and U.J. Krull*. A Novel Biochemical Sensor. *Chemtech*, **1992**, 22, 227-35.
 8. D.P. Nikolelis, J.D. Brennan, R.S. Brown, G. McGibbon and U.J. Krull*. Ion Permeability Through Bilayer Lipid Membranes For Biosensor Development: Control by Chemical Modification of Interfacial Regions Between Phase Domains. *Analyst*, **1991**, 116, 1221-1226.
 7. J.D. Brennan, R.S. Brown, S.C. Ferraro and U.J. Krull*. Hydronium Ion Sensitivity of Surface Stabilized Stearic Acid Membranes Prepared by Langmuir-Blodgett Monolayer Transfer. *Thin Solid Films*, **1991**, 203, 173-84.
 6. J.D. Brennan, R.S. Brown, D. Foster, R.K. Kallury and U.J. Krull*. Fluorescence Transduction of an Enzyme-Substrate Reaction by Covalently Immobilized Monolayers of Amphiphiles. *Analytica Chimica Acta*, **1991**, 255, 73-82.
 5. U.J. Krull*, D.P. Nikolelis, J.D. Brennan, R.S. Brown, M. Thompson, V. Ghaemmaghami and Kallury. Control of Ion Transport Through Lipid Membranes. *Analytical Proceedings*, **1991**, 28, 370-72.
 4. U.J. Krull*, J.D. Brennan, R.S. Brown, S. Hosein, B.D. Hougham and E.T. Vandenberg. Chemical Transduction with Fluorescent Lipid Membranes using Selective Interactions of Acetylcholine Receptor with Agonist/Antagonist and Acetylcholinesterase with Substrate. *Analyst*, **1990**, 115, 147-153.
 3. J.D. Brennan, R.S. Brown, C.P. McClintock and U.J. Krull*. Fluorescence Transduction of an Enzyme-Substrate Reaction by Modulation of Lipid Membrane Structure. *Analytica Chimica Acta*, **1990**, 237, 253-263.

Exhibit B

Section Title

PIN: 50862

John D. Brennan

2. U.J. Krull*, J. Brennan, R.S. Brown, G. McGibbon and K. Stewart. Chemical Sensors from Fluorometric Transduction of Selective Perturbations of Domain Structures within Lipid Multilayer Waveguides. *International Journal of Optoelectronics*, **1989**, 4, 133-146.
1. U.J. Krull*, J.D. Brennan, R.S. Brown and E.T. Vandenberg. Phase Structure Modulation of Fluorescent Lipid Membranes for Selective Chemical Transduction. *Proceedings of the Society of Photo-Optical Instrumentation Engineers (Optical Fibers in Medicine IV)*, **1989**, 1067, 53-60.

iii) Accepted for Publication

97. T.-Y. Lin, C.-H. Wu and J.D. Brennan*. Entrapment of Horseradish Peroxidase in Sugar-Modified Silica Monoliths: Toward the Development of a Biocatalytic Sensor. *Biosensors and Bioelectronics*, **2007**, accepted April 27, 2006.
98. S. Martic, N. Nagy, S. Ackloo, J.D. Brennan and M.A. Brook*. Towards the development of a covalently tethered MALDI system: A study of allyl-modified MALDI matrices. *Canadian Journal of Chemistry*, **2007**, accepted October 30, 2006.
99. J.D. Brennan. Biofriendly Sol-Gel Processing for Entrapment of Soluble and Membrane-Bound Proteins: Towards Novel Solid-Phase Assays for High-Throughput Screening. *Accounts of Chemical Research*, **2007**, accepted February 4, 2007. (**Invited Review Article**).

iv) Work Submitted For Publication

100. Y. Shen, G. Mackey, W. Chiuman, Y. Li* and J.D. Brennan*. Entrapment of Fluorescence-Signaling DNA Enzymes in Sol-Gel Derived Materials for Metal Ion Sensing. *Analytical Chemistry*, **2007**, submitted February 4, 2007.

v) Patents

1. J.D. Brennan and C.W.V. Hogue. Incorporation and Applications of Biomolecular Interactions within a Carrier. PCT/CA00/00779, US Patent Application No. 09/926,810, Filed June 23, 2000
2. Y. Li, J.D. Brennan and Z. Liu. Fluorescence-based Autocatalytic DNA Biosensor. US Patent Application No. 60/356,727, Filed February 18, 2003. PCT Application filed February 11, 2003.
3. M.A. Brook, J.D. Brennan and Y. Chen. Polyol-Modified Silanes as Precursors for Silica. U.S. Patent Application No. 10/449,511 and PCT application PCT/CA03/00790; Filed May 31, 2003.
4. Zheng Zhang, Yang Chen, John D. Brennan and Michael A. Brook, Methods and Compounds for Controlling the Morphology and Shrinkage of Silica Derived from Polyol-Modified Silanes. U.S. Patent Application and PCT Patent application PCT/CA03/01257; Filed August 23, 2003.
5. N. Rupcich and J.D. Brennan. Protein Microarray Technology for Assays Involving Coupled Reactions. U.S. Patent Application No. 10/698,492 and PCT Patent application PCT/CA03/01665; filed November 1, 2003.
6. T. Besanger, M.A. Brook and J.D. Brennan. Method of Immobilizing of Membrane-Bound Proteins. U.S. Patent Application No. 10/712,015 and PCT Patent application PCT/CA03/01757; filed November 14, 2003.
7. N. Rupcich, R. Nutiu, J.D. Brennan, Y. Li. Method of Immobilizing Nucleic Acid Aptamers. US Provisional Patent 60/545,525, filed February 11, 2004.
8. Zheng Zhang, Jorge A. Cruz-Aguado, Richard J. Hodgson, Dina Tleugabulova, John D. Brennan and Michael A. Brook. Protein Compatible Methods and Compounds for Controlling the Morphology and Shrinkage of Silica Derived from Polyol-Modified Silanes. U.S. Patent Application (Continuation in Part). Filed April 1, 2004.
9. J.D. Brennan, M.A. Brook and T.R. Besanger. Method of Immobilizing Membrane-Associated Molecules. Continuation in Part to Application 60/426,018, filed April 2, 2004.
10. T.R. Besanger, R.J. Hodgson, M.A. Brook and J.D. Brennan. Methods for Substrate and Inhibitor Screening Using Enzyme-Reactor Chromatography/Tandem Mass Spectrometry. PCT and US patents filed March 16, 2005.
11. H. Dong, M.A. Brook and J.D. Brennan. Methods for Forming Macroporous Monolithic Methylsilsesquioxanes. PCT and US patents filed April 29, 2005.

P. PRESENTATIONS AT MEETINGS (Speaker is Underlined, PI has asterisk)**i) Invited**

1. J.D. Brennan*. Entrapment of Proteins in Silica Materials for the Development of Bioanalysis Tools. 89th Canadian Society for Chemistry Conference, Halifax, NS, 2006. **(Award Lecture)**
2. N. Rupcich, R. Nutiu, Y. Li. and J.D. Brennan*. Entrapment of Fluorescent Signaling DNA Aptamers in Sol-Gel Derived Silica for the Development of Solid-Phase Enzyme Assays. 89th Canadian Society for Chemistry Conference, Halifax, NS, 2006. **(Invited)**
3. J.A. Cruz Aguado, X. Sui, M.A. Brook and J.D. Brennan*. Entrapment of Proteins in Silica Materials Derived from Polyol and Sugar-Modified Silane Precursors. 28th Australian Society for Biomaterials Conference, Rotorua, New Zealand, February, 2006. **(Keynote)**
4. J.D. Brennan*. Entrapment of Membrane-Bound Proteins into Biocompatible Sol-Gel Derived Materials for Bioaffinity Chromatography/Mass Spectrometry based Compound Screening. XIII International Workshop on Sol-Gel Science and Technology, Los Angeles, CA, 2005 **(Invited)**
5. J.D. Brennan*. Stabilizing Proteins by Entrapment into Biocompatible Silica Materials. Defense Advanced Research Projects Agency (DARPA), Fairfax, VA, 2005. **(Invited)**
6. J.D. Brennan*. Sol-Gel-Based Monolithic Columns as Enzyme Reactors and Affinity Supports for HTS. Canadian Proteome Society Meeting, London, ON, 2004. **(Invited)**
7. J.D. Brennan*; T.R. Covey; R.J. Hodgson; Y. Chen; Z. Zhang; D. Tleugabulova; X. Zhao; M. Organ; M.A. Brook and P. Kovarik. Frontal Affinity Chromatography/MS/MS (FAC/MS) High Throughput Screening (HTS) Using Sol-Gel Columns Containing Captured Protein Targets. 52nd American Society for Mass Spectrometry Conference, Nashville, TN, 2004. **(Invited)**
8. J.D. Brennan. Proteins Entrapped in Monolithic Silica Columns for HTS using FAC/MS. 87th Canadian Society for Chemistry Conference, London, ON, 2004. **(Invited)**
9. J.D. Brennan (2003). Entrapment of Functional Ligand-Gated Ion Channel Receptors in Sol-Gel Derived Silica: Applications in Biosensing and Drug Screening. ACS Northeastern Regional Meeting, Saratoga Springs, NY. **(invited)**
10. J.D. Brennan* (2003). Coupled Enzyme Reaction Microarrays based on Pin-Printing of Sol-Gel Biomaterials. Analytical Horizons Meeting, Ellecom, The Netherlands. **(Plenary Speaker)**.
11. J.D. Brennan* (2002). Optimizing Sol-Gel Entrapped Proteins for Bioanalytical Applications. 85th Canadian Society for Chemistry Conference, Vancouver, BC. **(invited)**.
12. K.K. Flora, G.L.G. Goring, M. Gulcev and J.D. Brennan* (2001). Designing New Materials for the Entrapment of Highly Active Biomolecules. FACSS Conference, Detroit, MI. **(invited)**
13. K.K. Flora, G.N. Bendiak, T. Keeling-Tucker and J.D. Brennan* (2001). Expanding the Utility of Sol-Gel Entrapped Proteins for Bioanalytical Applications. 84th Canadian Society for Chemistry Conference, Montreal, PQ. **(invited)**
14. J.D. Brennan* (2001). Designing New Materials for the Entrapment of Highly Active Biomolecules. 84th Canadian Society for Chemistry Conference, Montreal, PQ. **(invited)**
15. J.D. Brennan* (2000). Developing Bioanalytical Devices using Proteins Immobilized in Sol-Gel Derived Glass. DRES Biodetection Workshop, Medicine Hat, AL. **(Invited)**.
16. J.D. Brennan*, G.N. Bendiak, D. Benjamin, K. Flora, G.L.G. Goring, T. Keeling-Tucker and M. Rakic (2000). Developing Bioanalytical Devices using Proteins Immobilized in Sol-Gel Derived Glass. 83rd Canadian Society for Chemistry Conference, Calgary, AL. **(Invited)**.
17. J.D. Brennan* (1999). Development of fluorescence-based sensing strategies using analyte induced structural changes of sol-gel entrapped regulatory proteins. 82nd Canadian Society for Chemistry Conference, Toronto, ON. **(Invited)**.
18. J.D. Brennan* (1998). Controlling the performance of sol-gel entrapped proteins: Applications to biosensors. 44th International Conference on Analytical Sciences and Spectroscopy, Kingston, ON. **(Invited)**.
19. J.D. Brennan* (1997). A proposed model for the improved stability of proteins which are entrapped in TEOS derived glass matrices based on fluorescence studies of entrapped single Trp proteins. 80th Canadian Society for Chemistry Conference, Windsor, ON. **(Invited)**.
20. J.D. Brennan* (1996). Monitoring of tryptophan fluorescence to probe the structure and stability of single Trp proteins in porous glass matrices derived by the sol-gel method. 42nd International Conference on Analytical Sciences and Spectroscopy, London, ON. **(Invited)**

Exhibit B

Section Title

PIN: 50862

John D. Brennan

21. J.D. Brennan* (1996). Monitoring of tryptophan fluorescence to probe protein structure and stability in porous glass matrices derived by the sol-gel method. 79th Canadian Society for Chemistry Conference, St. John's, NFLD. **(Invited)**
22. J.D. Brennan, C.W.V. Hogue, B. Rajendran and A.G. Szabo* (1995). Fluorescence investigations of the denaturation of proteins containing 7-azatryptophan. 78th Canadian Chemical Congress Guelph, Ontario **(Invited)**.
23. J.D. Brennan, R.S. Brown, K.M.R. Kallury and U.J. Krull* (1994). Fluorimetric transduction of the urease-urea reaction and applications to biosensor development. 77th Canadian Chemical Congress, Winnipeg, Manitoba **(Invited)**.

ii) Contributed

24. J.D. Brennan. Solid-Phase HTS Using Chromatographic, Microarray and Aptamer Technologies. 5th Annual McMaster HTS Workshop, Hamilton, ON, 2006.
25. J. Sharma, D. Tleugabulova, W. Czardybon and J.D. Brennan*. Two-Site Ionic Labeling with Pyranine: Implications for Structural Dynamics Studies of Polymers and Polypeptides by Time-Resolved Fluorescence Anisotropy. 89th Canadian Society for Chemistry Conference, Halifax, NS, 2006.
26. J. Sharma, T. Besanger and J.D. Brennan* The Development of a Continuous Flow Competitive LC/MS Method for Screening Small Molecules Against Immobilized Receptors. 89th Canadian Society for Chemistry Conference, Halifax, NS, 2006.
27. I. Partserniak, T. Besanger, R. Hodgson and J.D. Brennan*. Evaluation of and Enzyme-Reactor Based High-Throughput Screening Technology for GSK3 Inhibitor Screening. 89th Canadian Society for Chemistry Conference, Halifax, NS, 2006.
28. Y. Li*, W. Chiuman, Y. Shen, S.A. Kandadai and J.D. Brennan. RNA-Cleaving Deoxyribozymes with Fluorescence-Signalling Capabilities. 89th Canadian Society for Chemistry Conference, Halifax, NS, 2006.
29. J.R.A. Green, N. Rupcich and J.D. Brennan*. The Development of a Nanovolume Cytochrome P450 Inhibition Assay using a Sol-Gel Derived Microarray. 89th Canadian Society for Chemistry Conference, Halifax, NS, 2006.
30. N. Rupcich, J.R.A. Green and J.D. Brennan*. Nanovolume detection of kinase inhibitors using a sol-gel derived multi-component protein microarray. The Ninth World Congress on Biosensors, Toronto, ON, 2006.
31. T.-Y. Lin, C.-H. Wu and J.D. Brennan*. Behavior of HRP-entrapped sugar-modified silica monoliths towards application as biocompatible minisensor. The Ninth World Congress on Biosensors, Toronto, ON, 2006.
32. Y. Shen, N. Rupcich, Y. Li and J.D. Brennan*. Investigation of Fluorescence-Signaling Deoxyribozymes as Biorecognition Elements for Bioactive Paper: Preliminary Studies on Immobilization and Fluorescence Detection of Aptazymes on Paper. 1st Annual SENTINEL Workshop, Guelph, ON, 2006.
33. H. Dong and J.D. Brennan. Polymerization of Methyltrimethoxysilane and Morphology Control Methylsilsesquioxane (MSQ) Using an Acid/Base Two-Step Procedure. XIII International Workshop on Sol-Gel Science and Technology, Los Angeles, CA, 2005
34. Peter Kovarik, Thomas R. Covey, Richard J. Hodgson, Michael A. Brook and John D Brennan*. Compound Screening using Capillary Scale Frontal Affinity Chromatography/MALDI Tandem Mass Spectrometry. 53rd American Society for Mass Spectrometry Conference, San Antonio, TX, 2005.
35. R.J. Hodgson, T.R. Besanger, M.A. Brook and J.D. Brennan*. Inhibitor Screening using Enzyme Reactor Chromatography/Tandem Mass Spectrometry. 53rd American Society for Mass Spectrometry Conference, San Antonio, TX, 2005.
36. J.D. Brennan, R.J. Hodgson, T.R. Besanger, Y. Chen, Z. Zhang, D. Tleugabulova, X. Zhao, M. Organ, M.A. Brook, T.R. Covey and P. Kovarik. HTS using Sol-Gel Based Monolithic Columns as Enzyme Reactors and Affinity Supports. 10th Society for Biomolecular Screening Conference, Orlando, FL, 2004.
37. N. Nagy, S. Ackloo, S. Martic, M.A. Brook and J.D. Brennan. Sol-Gel Derived Silica for MALDI/MS Applications. 31st FACSS Meeting, Portland, OR, 2004.

Exhibit B

Section Title

PIN: 50862

John D. Brennan

38. R.J. Hodgson, T.R. Besanger, Y. Chen, Z. Zhang, X. Zhao, M. Organ, M.A. Brook and J.D. Brennan. Monolithic Affinity Columns for LC/MS. 50th ICASS Conference, Halifax, NS, 2004.
39. D. Tleugabulova and J.D. Brennan. The Analytical Challenge of Time-Resolved Fluorescence Anisotropy: Understanding Molecular Motions in Complex Systems. 50th ICASS Conference, Halifax, NS, 2004.
40. J. Siu, D. Tleugabulova and J.D. Brennan. Evaluating Peptide-Silica Interactions using Time-Resolved Fluorescence Anisotropy. 50th ICASS Conference, Halifax, NS, 2004.
41. R.J. Hodgson, Y. Chen, Z. Zhang, D. Tleugabulova, X. Zhao, M. Organ, M.A. Brook, J.D. Brennan. Sol-Gel-Based Monolithic Affinity Columns for Nano-LC. HPLC 2004, Philadelphia, PA, 2004.
42. J. Cruz-Aguado, D.Y. Chen, Z. Zhang, M.A. Brook and J.D. Brennan*. Sugar-functionalized Silica Provides Enhanced Activity and Stability to Entrapped Enzymes. 87th CSC Conference, London, ON, 2004
43. N. Rupcich, R. Nutiua, Y. Li and J.D. Brennan.* Development of DNA Aptamer-Microarrays based Sol-Gel Derived Materials. 87th CSC Conference, London, ON, 2004.
44. T.R. Besanger and J.D. Brennan.* Entrapment of Highly Active Nicotinic Acetylcholine Receptor in Macroporous Sol-Gel Derived Materials. 87th CSC Conference, London, ON, 2004.
45. X. Sui, J. Cruz-Aguado, D.Y. Chen, M.A. Brook and J.D. Brennan.* Effect of Covalently Tethered Sugars on the Properties of Enzymes Entrapped in Sol-Gel Derived Silica. 87th CSC Conference, London, ON, 2004.
46. D. Tleugabulova, D.Y. Chen, Z. Zhang, M.A. Brook and J.D. Brennan*. Monitoring Solute Interactions with Modified Colloidal Silica Nanoparticles via Fluorescence Anisotropy Decay. 87th CSC Conference, London, ON, 2004.
47. J. Siu, D. Tleugabulova and J.D. Brennan. Evaluating Peptide-Silica Interactions using Time-Resolved Fluorescence Anisotropy. 87th CSC Conference, London, ON, 2004.
48. G.L.G. Goring and J.D. Brennan.* Preparation and Characterization of Sol-Gel Derived Biomolecular-Doped Films Suitable for Analytical Applications. 87th CSC Conference, London, ON, 2004.
49. D. Ilieva and J.D. Brennan*. Encapsulation of Dihydrofolate Reductase from E. Coli in Organically-Doped Silica Sol-Gels. 87th CSC Conference, London, ON, 2004.
50. Y. Gao and J.D. Brennan* (2003). The Development of New Sensing Methods Based on Analyte Induced Conformational Changes of Entrapped Regulatory Proteins. ACS Northeastern Regional Meeting, Saratoga Springs, NY.
51. G.L.G. Goring and J.D. Brennan* (2003). AFM Imaging and Time-Resolved Fluorescence Studies of Sol-Gel Derived Nanocomposite Materials Suitable for Biosensor Applications. ACS Northeastern Regional Meeting, Saratoga Springs, NY.
52. J.D. Brennan (2003). Biocompatible Materials for the Entrapment of Proteins: Applications in Bioaffinity Chromatography. XII International Workshop on Sol-Gel Science and Technology, Sydney, Australia.
53. T.R. Besanger and J.D. Brennan* (2003). Entrapment of Functional Ligand-Gated Ion Channel Receptors in Sol-Gel Derived Silica: Applications in Drug Screening. XII International Workshop on Sol-Gel Science and Technology, Sydney, Australia.
54. N. Rupcich and J.D. Brennan* (2003). Development and Optimization of Protein Microarrays based on Pin-Printing of Sol-Gel Entrapped Biomolecules. XII International Workshop on Sol-Gel Science and Technology, Sydney, Australia.
55. M.A. Brook*, Y. Chen and J.D. Brennan (2003). Sugar-Modified Silanes: Precursors for Silica Monoliths. XII International Workshop on Sol-Gel Science and Technology, Sydney, Australia.
56. Y. Chen, Z. Zhang, J.D. Brennan and M.A. Brook* (2003). A Glycerol-Derived Silica Precursor for the Encapsulation of Protein in Porous Silica Monoliths. XII International Workshop on Sol-Gel Science and Technology, Sydney, Australia.
57. G.L.G. Goring and J.D. Brennan* (2003) Time-Resolved Fluorescence Studies of Sol-Gel Derived Nanocomposite Materials Suitable for Biosensor Applications. 45th Rocky Mountain Conference on Analytical Chemistry, Denver, Colorado.
58. G.L.G. Goring and J.D. Brennan* (2003). Morphological Analysis of Sol-Gel Derived Nanocomposite Materials Suitable for Biosensor Applications. 45th Rocky Mountain Conference on Analytical Chemistry, Denver, Colorado.

Exhibit B

Section Title

PIN: 50862

John D. Brennan

59. Zhongjie Liu, Shirley H. J. Mei, John D. Brennan & Li, Y. (2002). Towards DNA enzyme biosensors. Ribo Club Meeting, Sherbrooke, Quebec.
60. Shirley H. J. Mei, Zhongjie Liu, John D. Brennan & Li, Y. (2002). A Fluorescing DNA enzyme. Ribo Club Meeting, Sherbrooke, Quebec.
61. M. Gulcev, K.K. Flora and J.D. Brennan* (2002). Improving the Properties of Sol-gel Entrapped Enzymes Using Glycerated Silane Precursors. 85th Canadian Society for Chemistry Conference, Vancouver, BC.
62. M. Gulcev and J.D. Brennan* (2002). Effect of Glycerated Silane Precursors on the Behaviour of Human Serum Albumin Entrapped in a Sol-Gel-Derived Glass. 85th CSC Conference, Vancouver, BC.
63. K.K. Flora, J.D. Brennan*, A. Capretta, K. Yong, D. Gerritsma and A. Jones (2002). Sensitization of Lanthanides by Non-Natural Amino Acids. 85th CSC Conference, Vancouver, BC.
64. K.K. Flora, J.D. Brennan, C.W.V. Hogue* and S.Sroka (2002). "Terbofluor": A Genetically Encoded Ultrasensitive Luminescent Protein Lanthanide Chelator. 85th CSC Conference, Vancouver, BC.
65. T. Besanger, Y. Zhang and J.D. Brennan* (2002). Sol-Gel Entrapment of Phospholipid Vesicles. 85th CSC Conference, Vancouver, BC.
66. T. Besanger and J.D. Brennan* (2002). Toward the Entrapment of Stable Transmembrane Proteins: Characterization of Gramicidin Entrapped into Sol Gel Derived Silicate Materials. 85th CSC Conference, Vancouver, BC.
67. N. Rupcich and J.D. Brennan* (2002). Use of Long Wavelength/Lifetime Probes for Fluorescence-Based Monitoring of Protein-Protein Interactions in Sol-Gel Derived Glasses. 85th CSC Conference, Vancouver, BC.
68. N. Rupcich and J.D. Brennan* (2002). Development of Protein-Microarrays Based on Pin-Spotting of Biomolecules Entrapped in Sol-Gel Derived Materials. 85th CSC Conference, Vancouver, BC.
69. G.L.G. Goring and J.D. Brennan* (2002). Fluorescence-Based Characterization of the Internal Environment of Sol-Gel Derived Nanocomposite Thin Films. 85th CSC Conference, Vancouver, BC.
70. G.L.G. Goring and J.D. Brennan* (2002). Examination of the Nanoscale Morphology of Protein-Doped Sol-Gel-Derived Thin Films using Tapping -Mode Atomic Force Microscopy. 85th CSC Conference, Vancouver, BC.
71. W. Jin, D. Chen, M.A. Brook* and J.D. Brennan* (2002). Development of Protein-Doped Sol-Gel Derived Materials Suitable for Bioaffinity Applications. 85th CSC Conference, Vancouver, BC.
72. Z. Liu, J.D. Brennan* and Y. Li* (2002). Fluorescence-Based Autocatalytic DNA Biosensor. 85th CSC Conference, Vancouver, BC.
73. K.K. Flora and J.D. Brennan* (2001). Effect of Aging on the Microenvironments of PRODAN Entrapped in a Tetraethylorthosilicate Derived Glass. 84th Canadian Society for Chemistry Conference, Montreal, PQ.
74. T. Keeling-Tucker, K.K. Flora and J.D. Brennan* (2001). Entrapment of Viable Protein-Protein Interaction Complexes in Sol-Gel Derived Matrixes. 84th Canadian Society for Chemistry Conference, Montreal, PQ.
75. W. Jin and J.D. Brennan* (2001). Properties of Antibodies Encapsulated within Modified Sol-Gel Glasses. 84th Canadian Society for Chemistry Conference, Montreal, PQ.
76. D.N. Benjamin, E.R. DiBattista and J.D. Brennan* (2001). Improving the Stability of Proteins Entrapped into Sol-Gel Derived Glass via Osmolyte Doping. Canadian Society for Chemistry Conference, Montreal, PQ.
77. G.L.G. Goring and J.D. Brennan* (2001). Fluorescence and Physical Characterization of Sol-Gel Derived Composite Biofilms. 84th Canadian Society for Chemistry Conference, Montreal, PQ.
78. M. Gulcev, K.K. Flora and J.D. Brennan* (2001). Improving the Stability of Sol-Gel Entrapped Enzymes Using Glycerated Silane Precursors. 84th Canadian Society for Chemistry Conference, Montreal, PQ.
79. D. Halilovic, K.K. Flora and J.D. Brennan* (2001). Development of a Fiber-Optic Ca(II) Sensor using a Fluorescently-Labelled Regulatory Protein in a Sol-Gel Derived Thin Film. 84th Canadian Society for Chemistry Conference, Montreal, PQ.
80. K. Flora and J.D. Brennan* (2000). Effect of Matrix Aging on the Thermodynamic Stability of a Biomolecule Entrapped in a Sol-Gel Derived Glass. 83rd Canadian Society for Chemistry Conference, Calgary, AL.

PIN: 50862

John D. Brennan

Section Title

PIN: 50862

- 19

April 27, 2007

Exhibit B

- Section Title PIN: 50862 John D. Brennan
100. W.J. Stevenson, J.D. Brennan, I.D. Clark, N.B. Adey, H.L. Hanson, B.K. Kay and A.G. Szabo* (1996). Characterization of calmodulin binding peptides using phage-display random peptide libraries. 40th Biophysical Society Meeting, Baltimore MD.
 101. B. Rajendran, J.D. Brennan, S. Cyr, D. Duforc, C.W.V. Hogue and A.G. Szabo* (1996). Fluorescence monitoring of the reversible unfolding of F102W and F102(7AW) rat parvalbumin in aqueous solution. 40th Biophysical Society Meeting, Baltimore MD.
 102. A.G. Szabo*, J.D. Brennan, C.W.V. Hogue and B. Rajendran (1996). Probing protein unfolding reactions using non-natural amino acids: An examination of wild type and W92(7AW) Tryptophanyl-tRNA synthetase using fluorescence techniques. 40th Biophysical Society Meeting, Baltimore MD.
 103. J.D. Brennan and A.G. Szabo* (1995). Photophysics of 7-azaindole in proton donating and accepting solvents. 39th Biophysical Society Meeting, San Francisco, CA.
 104. J.D. Brennan, S. Anderson, C.W.V. Hogue, B. Rajendran and A.G. Szabo* (1995). Enzymatic resolution of D and L enantiomers of 7-azatryptophan and enzymatic synthesis of N-acetyl-L-7-azatryptophanamide. 39th Biophysical Society Meeting, San Francisco, CA.
 105. J.D. Brennan, C.W.V. Hogue, A. Ito, L. Juliano and A.G. Szabo* (1995). Interaction of enantiomers of lysyl-7-azatryptophyl-lysine with acidic phospholipid vesicles: A fluorescence study. 78th Canadian Chemical Congress, Guelph, Ontario.
 106. J.D. Brennan, C.W.V. Hogue, B. Rajendran and A.G. Szabo* (1995). Fluorescence investigations of the denaturation of surface-bound proteins containing 7-azatryptophan. 41st International Conference on Analytical Sciences and Spectroscopy, Windsor, Ontario.
 107. J.D. Brennan and A.G. Szabo* (1994). Photophysics of 7-azatryptophan: Evidence for an excited state reaction. 38th Biophysical Society Meeting, New Orleans, LA.
 108. J.D. Brennan, C.W.V. Hogue, A. Ito, L. Juliano and A.G. Szabo* (1994). Time resolved fluorescence of enantiomers of model peptides containing 7-azatryptophan. 38th Biophysical Society Meeting, New Orleans, LA.
 109. J.D. Brennan, N. Adey, I.D. Clark, C.W.V. Hogue, B. Kay and A.G. Szabo* (1994). Characterization of the calmodulin binding of a 22 mer peptide from a M13 phage library. 38th Biophysical Society Meeting, New Orleans, LA.
 110. J.D. Brennan, C.W.V. Hogue and A.G. Szabo* (1994). Photophysics of 7-azatryptophan: An intrinsic probe of protein folding. 77th Canadian Chemical Congress, Winnipeg, Manitoba.
 111. J.D. Brennan, R.S. Brown and U.J. Krull* (1993). A model for self-quenching of nitrobenzoxadiazole in lipid membranes. 76th Canadian Chemical Congress, Sherbrooke, Quebec.
 112. J.D. Brennan, R.S. Brown, K.M.R. Kallury and U.J. Krull* (1993). Fluorescence investigations of chemically induced structural alterations in self-assembled membranes of ionizable alkylsilanes. 67th Symposium on Surface and Colloid Science, American Chemical Society, Toronto, Ontario.
 113. J.D. Brennan, R.S. Brown, A. Della Manna, P. Piuino, K.M.R. Kallury and U.J. Krull* (1993). Transduction of the reaction between urea and covalently immobilized urease by fluorescent amphiphilic membranes. Sixth International Conference on Organized Molecular Films (LB6), Trois-Rivières, Quebec.
 114. J.D. Brennan, D.P. Nikolelis, S. Seethaler and U.J. Krull* (1993). Bilayer lipid membranes as electrochemical switches in reactions involving alterations of surface charge. Sixth International Conference on Organized Molecular Films (LB6), Trois-Rivières, Quebec.
 115. J.D. Brennan, R.S. Brown, A. Della Manna, K.M.R. Kallury and U.J. Krull* (1992). Covalent immobilization of amphiphile monolayers onto optical fibers for fluorimetric detection of urea. 75th Canadian Chemical Congress, Edmonton, Alberta.
 116. J.D. Brennan, R.S. Brown, J. Cirello, H. Cohen, A. Fournier and U.J. Krull* (1992). Fluorescence investigations of the complexation of antifusilade antibody with labelled fusilade for homogeneous immunoassay development. 75th Canadian Chemical Congress, Edmonton, Alberta.
 117. J.D. Brennan, R.S. Brown, K.M.R. Kallury and U.J. Krull* (1991). Fluorescence transduction of enzyme-substrate reactions by covalently immobilized amphiphilic membranes. 74th Canadian Chemical Congress, Hamilton, Ontario.
 118. J.D. Brennan, R.S. Brown, S. Ferraro and U.J. Krull* (1990). Modification of the structure of fluorescent lipid and fatty acid monolayers by pH and lateral pressure alterations. 33rd IUPAC Meeting, International Symposium on Macromolecules, Montreal, Quebec.

Exhibit B

Section Title

PIN: 50862

John D. Brennan

119. J.D. Brennan, C.P. McClintock and U.J. Krull* (1989). Mechanistic aspects of fluorescence transduction of an enzyme-substrate reaction by lipid and fatty acid membranes. 73rd Canadian Chemical Congress, Halifax, Nova Scotia.
120. J.D. Brennan, R.S. Brown and U.J. Krull* (1989). The potential of fluorescent lipid membranes as transducers in biosensors. Symposium on Biosensors, American Chemical Society, Chapel Hill, North Carolina.

iii) Invited Seminars Presented at Other Institutions

1. J.D. Brennan. "Entrapment of Proteins and Functional Nucleic Acids in Silica Materials for the Development of Bioanalysis Tools". Simon Fraser University, Burnaby, BC, September 13, 2006.
2. J.D. Brennan. "Entrapment of Proteins in Silica Materials for the Development of Bioanalysis Tools" Concordia University, Montreal, March 11, 2006. (**James Dick Lecturer**)
3. J.D. Brennan. "Entrapment of Proteins in Biocompatible Silica" Flinders University, Adelaide, Australia, February 15, 2006.
4. J.D. Brennan. "Fluorescence Methods for Examining Sol-Gel Materials" Flinders University, Adelaide, Australia, February 14, 2006.
5. J.D. Brennan. "Entrapment of Proteins in Silica Materials for the Development of Bioanalysis Tools" University of Melbourne, Melbourne, Australia, February 13, 2006.
6. J.D. Brennan. "Compound Screening by Affinity Chromatography/Mass Spectrometry Using Monolithic Bioaffinity Columns" Schering-Plough Research Institute, Boston, MA, September 25, 2005.
7. J.D. Brennan. "Optimizing Sol-Gel Derived Biomaterials for Chromatographic and Sensor Applications" Department of Chemistry, University of Saskatchewan, September 28, 2004.
8. J.D. Brennan. "Optimizing Sol-Gel Entrapped Proteins for Bioanalytical Applications". Department of Chemistry, University of Toronto, November 4, 2003.
9. J.D. Brennan. "Optimizing Sol-Gel Entrapped Proteins for Bioanalytical Applications". BD Biosciences, Raleigh, NC, November 15, 2002.
10. J.D. Brennan. "Optimizing Sol-Gel Entrapped Proteins for Bioanalytical Applications". MDS-Sciex, Concord, ON, April 27, 2002.
11. Y. Li, E.D. Brown and J.D. Brennan*. "Aptamer Technologies for Proteomics Applications". Genome Prairie Workshop, August 30, 2001
12. Y. Li and J.D. Brennan*. "Evaluating DNA Aptamers as Fluorescent Sensors for Toxic Substances". Defence Research Establishment at Suffield, Suffield, AL, August 16, 2001
13. Y. Li and J.D. Brennan*. "Aptamer Technologies for Biosensor and Drug Screening Applications". MDS-Sciex, Concord, ON, August 14, 2001
14. J.D. Brennan*. "Developing Bioanalytical Devices using Proteins Immobilized in Sol-Gel Derived Glass". Department of Chemistry, University of Western Ontario, September 27, 2000.
15. J.D. Brennan*. "Developing Bioanalytical Devices using Proteins Immobilized in Sol-Gel Derived Glass". Becton Dickinson Biosciences, Rayleigh, NC, September 21, 2000.
16. J.D. Brennan*. "Developing Bioanalytical Devices using Proteins Immobilized in Sol-Gel Derived Glass". Department of Chemistry, University of Calgary, August 11, 2000.
17. J.D. Brennan*. "Developing Bioanalytical Devices using Proteins Immobilized in Sol-Gel Derived Glass". Department of Chemistry, University of Alberta, August 10, 2000.
18. J.D. Brennan*. "Developing Bioanalytical Devices using Proteins Immobilized in Sol-Gel Derived Glass". Department of Chemistry, University of Waterloo, July 27, 2000.
19. J.D. Brennan*. "Development of fluorescence-based assays for compounds that disrupt protein-protein interactions". MDS-Proteomics, June 16, 2000.
20. J.D. Brennan*. "Development of fluorescence-based assays for compounds that disrupt protein-protein interactions". Samuel Lunenfeld Research Institute, November 4, 1999.
21. J.D. Brennan*. "Using Sol-Gel Entrapped Proteins for the Development of Optical Biosensors". Department of Chemistry, Saint Mary's University, October 1, 1999.
22. J.D. Brennan*. "Controlling the performance of sol-gel entrapped proteins: Applications to biosensors". Department of Chemistry, McMaster University, May 22, 1998.
23. J.D. Brennan*. "Controlling the performance of sol-gel entrapped proteins: Applications to biosensors". Department of Chemistry and Biochemistry, University of Windsor, May 6, 1998.

Exhibit B

Section Title

PIN: 50862

John D. Brennan

24. J.D. Brennan*. "Controlling the performance of sol-gel entrapped proteins: Applications to biosensors". Samuel Lunenfeld Research Institute, Mount Sinai Hospital, April 30, 1998.
25. J.D. Brennan*. "Controlling the performance of sol-gel entrapped proteins: Applications to biosensors". Department of Food Science, University of Guelph, April 24, 1998.
26. J.D. Brennan*. "Controlling the performance of sol-gel entrapped proteins: Applications to biosensors". Department of Chemistry, Queen's University, March 25, 1998.
27. J.D. Brennan*. "Enhancing the stability of proteins by entrapment in sol-gel derived glass matrices: Application to improved optical biosensors". Department of Chemistry, University of Toronto, February 13, 1997.
28. J.D. Brennan*. "Fluorescence investigations of the thermal and chemical stability of proteins containing 7-azatryptophan." Department of Chemistry, State University of New York at Buffalo, January 5, 1996.

Q. ADMINISTRATIVE RESPONSIBILITIES

i) Departmental

A) Brock University (All administrative responsibilities are listed)

1. Member of the Technical Services Committee (1995-1998)
2. Faculty Advisor to Brock University Chemistry Society and for the Canadian Institute of Chemistry (1995-1997).
3. Co-ordinator of the fourth year research course CHEM/BTEC 4F90/4F91 (1995-1997)
4. Co-ordinator of the summer research courses CHEM 2P98 and CHEM 3P98 (3 students).
5. Dean's Delegate on M.Sc. Thesis Defense for Natalie Limoges, October 1995.
6. Member of B.Sc. research committee for a total of 11 students.
7. Member of M.Sc. committee for a total of 5 students.
8. Organized a Seminar Series for the Department of Chemistry which ran weekly throughout the Winter Term (January to May).
9. Judge at Niagara Regional Science and Engineering Fair, 1996-1997.
10. Organized participation of 4th year students in the Southwestern Ontario Undergraduate Student's Conferences in 1996 and 1997.
11. Helped to organize National Chemistry Week events in conjunction with members of the Brock University Chemistry Club (1995-1997).
12. Supervised Giles Holland as part of a high school OAC level independent study course.
13. Supervised Greg Tkaczyk as part of the Niagara Region Mentorship program.
14. Organized a workshop for Scientifically Yours, May 1-3, 1996.
15. Organized and presented a workshop in the Niagara-Brock Science Interface program, May 22, 1996.
16. Performed duties as a Bedel at Brock University Convocation Ceremony, June 14, 1996.
17. Organized a workshop for CHEM and BTEC fourth year Honours students covering NSERC and OGS application procedures, benefits of graduate study and grad school application procedures, Sept 20, 1996.
18. Member of Departmental Committee for Ranking of NSERC scholarship applicants, Fall 1996.
19. Member of Departmental Committee for Ranking of OGS scholarship applicants, Fall 1996.
20. Member of the OGS provincial ranking committee, February, 1997.
21. On the active reviewers list for the following journals:
 - Canadian Journal of Chemistry
 - Canadian Journal of Biochemistry and Cell Biology
 - Applied Biochemistry and Biotechnology
22. Organized and presented a workshop for 3rd year students on the requirements and expectations for the CHEM and BTEC 4F90 / 4F91 research and thesis courses, March 20, 1997. A total of 33 students and 8 faculty members attended.
23. Represented Brock University at the Environmental Sciences and Technology Alliance of Canada (ESTAC) Visioning Workshop, April 8-10, 1997, Mississauga, Ontario.
24. Organized and presented a workshop in the Niagara-Brock Science Interface program, May 21, 1997.

Exhibit B

Section Title

PIN: 50862

John D. Brennan

25. Organized a workshop for CHEM and BTEC fourth year Honours students covering NSERC and OGS application procedures, benefits of graduate study and grad school application procedures, October 1, 1997. (Note: 2 chemistry students received NSERC graduate scholarships in 1998).
26. Reviewed four research grants for Natural Sciences and Engineering Research Council of Canada (GSC 026).
27. Helped organized a workshop for 3rd year students on the requirements and expectations for the CHEM and BTEC 4F90 / 4F91 research and thesis courses, March 23, 1998. A total of 20 students and 8 faculty members attended.
28. Judged at Niagara Regional Science and Engineering Fair, April 8, 1998.
29. Supervised Bryon Jeremy Frid as part of the Niagara Regional Mentorship program.

B) McMaster University

i) Departmental Responsibilities

McMaster Chemistry Department Seminar Co-ordinator, 1998-2000.
Graduate Admissions Committee, 1999-2001.
Graduate Curriculum Committee, 1999-2001.
Head of Graduate Student Recruiting, 1999-2001.
Chair's Advisory Committee (January 2000 – 2002, 2005-2006).
Chair – General Instrument Facility Committee (2000 – 2003).
Chair – Graduate Recruiting committee (2004-2006).
Chair – Chemical Biology committee – 2005/2006.
Director – Chemical Biology Graduate Program – 2006 – present.

ii) Faculty Responsibilities

Faculty of Science Graduate Curriculum, Policy, Admissions and Study Committee.
Dean of Science search committee, 2005.

iii) University Responsibilities

None

R. BIOGRAPHICAL LISTINGS

i) Active Collaborations

a) Internal

1. Prof. Michael Brook. Development of new silica precursors for sol-gel based protein entrapment.
2. Profs. Gerry Wright and Eric Brown. Assay development for high-throughput screening.
3. Prof. Yingfu Li. Fluorescent DNA Aptamers for biosensors and drug screening.
4. Prof. Alfredo Capretta. Chemical Biology Studies of Glycogen Synthase Kinase 3.

b) External

5. Prof. Frank V. Bright, State University of New York at Buffalo. Picosecond fluorescence spectroscopy of proteins.
6. Prof. Janusz Pawliszyn, University of Waterloo. Selective solid-phase microextraction using protein doped sol-gel films.
7. Drs. Christopher W.V. Hogue, Anthony J. Pawson and Frank Sicheri, Samuel Lunenfeld Research Institute. Fluorescence studies of Tb(III) binding proteins and protein-protein interactions.
8. Dr. Daniel Figeys, MDS-Ocata Proteomics. Development of sol-gel entrapped protein arrays and interfacing with microfluidic devices.
9. Prof. Michael Organ, York University and Dr. David Schreimer, INH Technologies. Development of high-throughput screening technologies based on frontal-affinity chromatography interfaced to a mass spectrometer.

Exhibit B

Section Title

PIN: 50862

John D. Brennan

10. Dr. John Beutler, National Institutes of Health. Development of monolithic columns containing HIV Reverse Transcriptase for HTS.
11. Dr. Dev Pinto, NRC Institute for Marine Biosciences. Development of monolithic columns.
12. Dr. Deitrich Volmer, NRC Institute for Marine Biosciences. Development of monolithic columns.

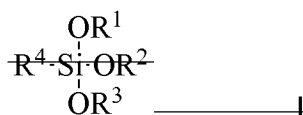
S. OTHER RESPONSIBILITIES

30. Member of Ph.D. supervisory committee for Suzanne Ackloo, Anna Trikoplous, Anna Shulkin, Ming Li, Tariq Mukhtar, Amro Ragheb and Razwan Nutiu, William Chiuman (current), Chand Magrit (Current), Tushar (current), Weihang Zhao (current), Lucy Ye (current).
31. Member of M.Sc. supervisory committee for, David Badurina, Shirley Mei, Giselle Segui-Lines (current).
32. Member of Ph.D. Examination committee for Philip van Beynen, November 4, 1998.
33. Member of Ph.D. Examination committee for Xiucheng Wu, May 10, 1999.
34. Ph.D. External examiner for Chiciu Liu, University of Western Ontario, July 15, 1999.
35. Member of Ph.D. Examination committee for Hany Aziz, August 27, 1999.
36. Member of M.Sc. Examination committee for Genevieve Jones, December 17, 1999.
37. Member of Ph.D. Examination committee for Byron De Labarge, April 4, 2000.
38. Member of Ph.D. Examination committee for Matt Pearson, August 18, 2000.
39. Member of Ph.D. Examination committee for Cecile Marczinski, June 18, 2001.
40. Member of M.Sc. Examination committee for David Badurina, March 18, 2002.
41. Member of Ph.D. Examination committee for Jonathon Millman, June 28, 2003.
42. Member of M.Sc. Examination committee for Shirley Mei, September 18, 2003.
43. Member of Ph.D. Examination committee for Kari-Ann Draker, October 8, 2003.
44. Ph.D. External Examiner for Donald Andrew James, University of Toronto, November 4, 2003.
45. Ph.D. External Examiner for Soma Sharma, Banduras Hindu University, June 2004.
46. Member of Ph.D. Examination committee – Sept 2004.
47. Chair, Ph.D. Examination committee for Young-Min Kim, Jan 25, 2005.
48. Member of Ph.D. Examination committee for Amit Bhavsar, July 25, 2006.
49. Ph.D. External Examiner for Lisa Elizabeth Rodgers, “The Molecular Characterization of Sol-Gel Biocatalysts” University of New South Wales, September 2006.
50. Ph.D. External Examiner for Bhaskar Murari, “A Novel Sol-Gel Thin Film Based Fluorescence Technique for Quantitation of Myoglobin: A Cardiac Marker.” Indian Institute of Technology, October 2006.

Exhibit C

Listing of Claims:

1. (Currently amended) A method of preparing meso- and macroporous siliceous materials comprising combining an organic polyol silane precursor with one or more additives under conditions suitable for hydrolysis and condensation of the precursor to a siliceous material and for phase separation to occur before gelation, wherein the one or more additives are selected from one or more water-soluble polymers and ~~one or more trifunctional silanes of Formula I:~~



~~wherein OR¹, OR² and OR³ are the same or different and represent a group that is hydrolyzed under normal sol-gel conditions to provide Si-OH groups; and R⁴ is group that is not hydrolyzed under normal sol-gel conditions, wherein the conditions suitable for hydrolysis and condensation of the precursor to a siliceous material comprise combining the organic polyol silane precursor with the one or more additives at a pH in the range of about 4 to 10 about 11.5.~~

2. (Original) The method according to claim 1, wherein the one or more additives are water soluble polymers selected from one or more of polyethers, polyalcohols, polysaccharides, poly(vinyl pyridine), polyacids, polyacrylamides and polyallylamine.

3. (Original) The method according to claim 2, wherein the one or more additives are water soluble polymers selected from one or more of polyethylene oxide (PEO), polyethylene glycol (PEG), amino-terminated polyethylene oxide (PEO-NH₂), amino-terminated polyethylene glycol (PEG-NH₂), polypropylene glycol (PPG), polypropylene oxide (PPO), polypropylene glycol bis(2-amino-propyl ether) (PPG-NH₂), polyvinyl alcohol, poly(acrylic acid), poly(vinyl pyridine), poly(N-isopropylacrylamide) (polyNIPAM) and polyallylamine (PAM).

Exhibit C

4. (Original) The method according to claim 3, wherein the one or more additives are water soluble polymers selected from one or more of PEO, PEO-NH₂, PEG, PPG-NH₂, polyNIPAM and PAM.
5. (Original) The method according to claim 3, wherein the one or more additives are water soluble polymers selected from one or more of PEO, PEO-NH₂ and polyNIPAM.
6. (Original) The method according to claim 1, wherein the one or more additives is a mixture of water soluble polymers,
7. (Original) The method according to claim 6 wherein the mixture of water soluble polymers comprises PEO and PEO-NH₂.
8. (Original) The method according to claim 5, wherein the one or more additives is PEO.
9. (Original) The method according to claim 8, wherein the PEO has a molecular weight that is greater than about 10,000 g/mol.
10. (Original) The method according to claim 9, wherein the PEO is used at a concentration of greater than about 0.005 g/mL of final solution.
11. (Original) The method according to claim 5, wherein the one or more additives is PEO-NH₂.
12. (Original) The method according to claim 11, wherein the PEO-NH₂ has a molecular weight that is greater than about 3,000 g/mol and is used at a concentration of about 0.005 g/mL of final solution.

Exhibit C

13. (Original) The method according to claim 5, wherein the one or more additives is poly(N-isopropylacrylamide).

14. (Original) The method according to claim 13, wherein the poly(N-isopropylacrylamide) has a molecular weight that is about 10,000 g/mol and is used at a concentration of about 0.005 g/mL of final solution.

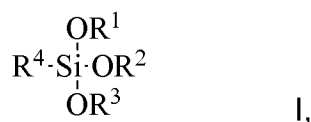
15. – 36. (Cancelled herein)

37. (Original) The method according to claim 1, wherein the organic polyol silane precursor is selected from the group consisting of diglycerylsilane (DGS), monosorbitylsilane (MSS), monomaltosylsilane (MMS), dimaltosylsilane (DMS) and dextran-based silane (DS).

38. (Currently amended) The method according to claim 1, wherein the ~~conditions suitable for the hydrolysis and condensation of the precursor to a siliceous material include a pH in the range of about 4-11.5~~ comprise combining the organic polyol silane precursor with the one or more additives in aqueous solutions and with optional sonication to assist in dissolution.

39. (Previously amended) A method of preparing siliceous materials with low shrinkage characteristics comprising:

(a) combining an aqueous solution of one or more compounds of Formula I:



wherein OR¹, OR² and OR³ are the same or different and represent a group that is hydrolyzed under normal sol-gel conditions to provide Si-OH groups; and R⁴ is group that is not hydrolyzed under normal sol-gel conditions, with an aqueous solution of an organic polyol silane precursor;

(b) adjusting the pH of the solution in (a) to about 4-11.5;

Exhibit C

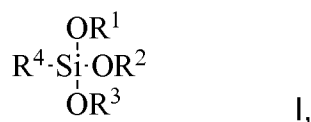
(c) allowing the solution of (b) to gel;

(d) aging the gel of (c); and

(e) drying the aged gel in air.

40. (Original) A siliceous material prepared using the method according to claim 1.

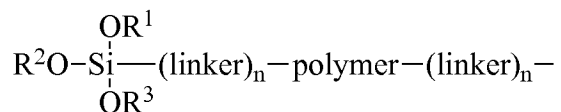
41. (Currently amended) A method of preparing monolithic meso- and macroporous silica materials comprising combining an organic polyol silane precursor with one or more additives selected from one or more water-soluble polymers and one or more compounds of Formula I:



wherein OR^1 , OR^2 and OR^3 are the same or different and represent a group that is hydrolyzed under normal sol-gel conditions to provide Si-OH groups, R^4 is group selected from polymer-(linker)_n- and

$\begin{array}{c} \text{OR}^1 \\ | \\ \text{R}^2\text{O-Si-} \\ | \\ \text{OR}^3 \end{array} \text{-(linker)}_n\text{-polymer-(linker)}_n\text{-}$ and $n = 0-1$, under conditions suitable for hydrolysis and condensation of the precursor to a siliceous material and for ~~where a phase transition to~~ occurs before gelation, wherein the conditions where a phase transition occurs before gelation comprise combining the organic polyol silane precursor with the one or more additives at a pH in the range of about 4 to 10 about 11.5.

42. (Original) The method according to claim 41, wherein R^4 is



43. (Original) The method according to claim 42, wherein the linker group is a C₁₋₄alkylene group and n is 1.

Exhibit C

44. (Original) The method according to claim 42, wherein OR¹, OR² and OR³ are the same and are selected from C₁₋₄alkoxy.

45. (Original) The method according to claim 42, wherein the polymer is PEO.

46. (Original) The method according to claim 41 wherein the compound of Formula I is selected from the group consisting of:

(CH₂CH₂O)_p[(EtO)₃Si(C₃H₆)]₂, p ~4-5, average MW 200 (Compound **5a**);

(CH₂CH₂O)_p[(EtO)₃Si(C₃H₆)]₂, p ~13, average MW 600 (Compound **5b**);

(CH₂CH₂O)_p[(EtO)₃Si(C₃H₆)]₂, p ~44, average MW 2000 (Compound **5c**); and

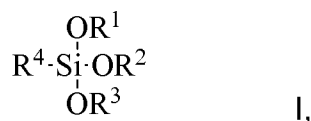
(CH₂CH₂O)_p[(EtO)₃Si(C₃H₆)]₂, p ~227, average MW 10,000 (Compound **5d**).

47. (Original) The method according to claim 41, wherein the water soluble polymer is selected from one or more of PEO, PEO-NH₂ and poly(NIPAM).

48. (Original) A meso/macroporous silica monolith prepared using the method according to claim 41.

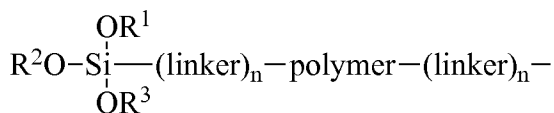
49.-53. (Cancelled herein)

54. (Currently amended) A method of preparing a meso- and macroporous monolithic silica chromatographic column comprising placing a solution comprising an organic polyol silane precursor and one or more additives selected from one or more water-soluble polymers and one or more compounds of Formula I:



wherein OR¹, OR² and OR³ are the same or different and represent a group that is hydrolyzed under normal sol-gel conditions to provide a Si-OH group; R⁴ is group selected from polymer-(linker)_n- and

Exhibit C

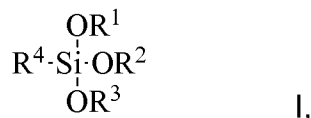


and $n = 0-1$, in a column under conditions

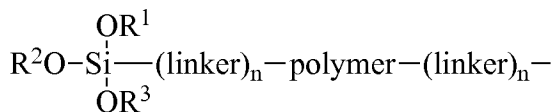
suitable for hydrolysis and condensation of the precursor to a siliceous material and for a phase transition to occur before gelation, wherein the conditions ~~suitable for a phase transition to occur before gelation~~ comprise combining the organic polyol silane precursor with the one or more additives at a pH in the range of about 4 to 10 ~~about 11.5~~.

55. (Previously amended) The method according to claim 54, wherein the solution further comprises one or more substances, which provide cationic sites that counterbalance an anionic charge of the silica to reduce non-selective interactions

56. (Currently amended) A chromatographic column comprising a meso- and macroporous silica monolith prepared by combining an organic polyol silane precursor and one or more additives selected from one or more water-soluble polymers and one or more compounds of Formula I:



wherein OR^1 , OR^2 and OR^3 are the same or different and represent a group that is hydrolyzed under normal sol-gel conditions to provide Si-OH groups; R^4 is group selected from polymer-(linker)_n- and



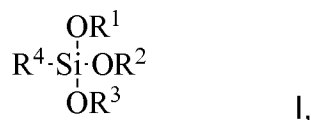
and $n = 0-1$, under conditions suitable for

hydrolysis and condensation of the precursor to a siliceous material and for ~~where a phase transition to occurs before gelation~~, wherein the conditions ~~suitable for a phase transition to occur before gelation~~ comprise combining the organic polyol silane precursor with the one or more additives at a pH in the range of about 4 to 10 ~~about 11.5~~.

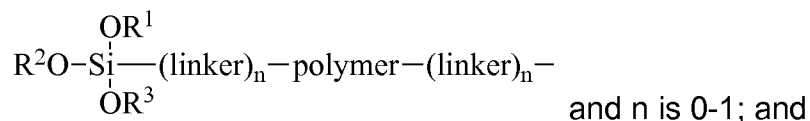
Exhibit C

57. (Currently amended) A method of preparing a meso- and macroporous monolithic silica column having an active biomolecule entrapped therein comprising combining:

- a) a polyol-silane derived silica precursor;
- b) one or more additives selected from one or more water soluble polymers and one or more compounds of Formula I:



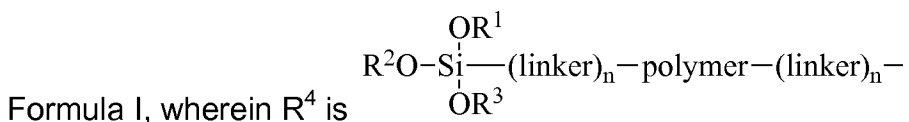
wherein OR^1 , OR^2 and OR^3 are the same or different and represent a group that is hydrolyzed under normal sol-gel conditions to provide Si-OH groups, R^4 is group selected from polymer-(linker)_n- and



- c) a biomolecule;

under conditions suitable for hydrolysis and condensation of the precursor to a siliceous material and for wherein a phase separation to occurs before gelation, wherein the conditions ~~suitable for a phase transition to occur before gelation~~ comprise combining the organic polyol silane precursor with the one or more additives at a pH in the range of about 4 to 10 ~~about 11.5~~.

58. (Original) The method according to claim 57, wherein the one or more additives is one or more water soluble polymers or one or more compounds of



59. (Previously amended) The method according to claim 57, wherein the organic polyol silane silica precursor, one or more additives and biomolecules are also combined with a substance which provides cationic sites that counterbalance an anionic charge of the silica to reduce non-selective interactions.

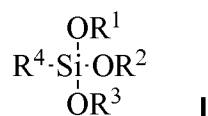
Exhibit C

60. (Original) A chromatographic column prepared using a method according to claim 57.

61. (Original) A method of performing immunoaffinity chromatography, sample cleanup, solid phase extraction or preconcentration of analytes, removal of unwanted contaminants, solid phase catalysis or frontal affinity chromatography comprising:

- (a) applying a sample to a column according to claim 60: and
- (b) performing immunoaffinity chromatography, sample cleanup, solid phase extraction or preconcentration of analytes, removal of unwanted contaminants, solid phase catalysis or frontal affinity chromatography.

62. (Previously amended) A method of preparing siliceous materials with enhanced protein stabilizing ability comprising combining an organic polyol silane precursor with one or more additives under conditions suitable for hydrolysis and condensation of precursor to a siliceous material, wherein the one or more additives is selected from one or more trifunctional silanes of Formula I:



wherein wherein OR^1 , OR^2 and OR^3 are the same or different and represent a group that is hydrolyzed under normal sol-gel conditions to provide a Si-OH group and R^4 is polyol-(linker)-.

63. (Previously amended) The method according to claim 62, wherein the polyol in R^4 is derived from sugar alcohols, sugar acids, saccharides, oligosaccharides or polysaccharides.

64. (Original) The method according to claim 63, wherein the polyol in R^4 is derived from allose, altrose, glucose, mannose, gulose, idose, galactose, talose, ribose, arabinose, xylose, lyxose, threose, erythrose, glyceraldehydes, sorbose,

Exhibit C

fructose, dextrose, levulose, sorbitol, sucrose, maltose, cellobiose, lactose, dextran (500-50,000 MW), amylose, pectin, glycerol, propylene glycol or trimethylene glycol.

65. (Original) The method according to claim 64, wherein the polyol in R⁴ is derived from glycerol, sorbitol, maltose, trehalose, glucose, sucrose, amylose, pectin, lactose, fructose, dextrose or dextran.

66. (Original) The method according to claim 65, wherein the polyol in R⁴ is derived from glycerol, sorbitol, glucose, maltose or dextran.

67. (Original) The method according to claim 66, wherein the polyol in R⁴ is derived from glucose or maltose.

68. (Previously amended) The method according to claim 62 wherein the one or more additives is GluconamideSi (Compound **1**) and/or MaltonamideSi (Compound **2**).

69. (Original) The method according to claim 62, wherein the protein is a kinase, luciferase, or urease or is Factor Xa.

70. (Original) The method according to claim 69, wherein the protein is Src protein tyrosine kinase.

71. (Original) The method according to claim 62, further comprising combining the organic polyol silane precursor and one or more additives with a substrate for the protein to be entrapped.

72. (Original) The method according to claim 71, wherein the protein is a kinase and the substrate is a source of phosphate.

Exhibit C

73. (Original) The method according to claim 72, wherein the substrate is ATP.

74. (Previously added) The method according to claim 59, wherein the substance which provides cationic sites that counterbalance an anionic charge of the silica to reduce non-selective interactions is aminopropyltriethoxysilane (APTES), PAM, PPG-NH₂ and/or PEG-NH₂.

75. (New) The method according to claim 39, wherein OR¹, OR² and OR³ are the same or different and are derived from organic di- or polyols.

76. (New) The method according to claim 75, wherein OR¹, OR² and OR³ are the same or different and are derived from sugar alcohols, sugar acids, saccharides, oligosaccharides or polysaccharides.

77. (New) The method according to claim 75, wherein OR¹, OR² and OR³ are the same or different and are derived from allose, altrose, glucose, mannose, gulose, idose, galactose, talose, ribose, arabinose, xylose, lyxose, threose, erythrose, glyceraldehydes, sorbose, fructose, dextrose, levulose, sorbitol, sucrose, maltose, cellobiose, lactose, dextran (500-50,000 MW), amylose, pectin, glycerol, propylene glycol or trimethylene glycol.

78. (New) The method according to claim 77, wherein OR¹, OR² and OR³ are the same or different and are derived from glycerol, sorbitol, maltose, trehalose, glucose, sucrose, amylose, pectin, lactose, fructose, dextrose and dextran.

79. (New) The method according to claim 77, wherein OR¹, OR² and OR³ are the same or different and are derived from glycerol, sorbitol, maltose or dextran.

Exhibit C

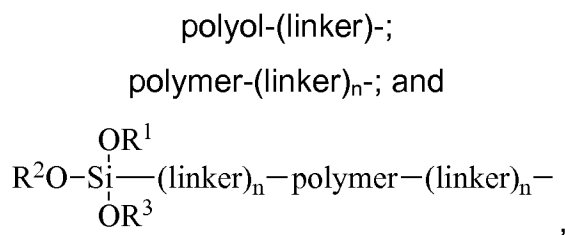
80. (New) The method according to claim 39, wherein OR^1 , OR^2 and OR^3 are the same or different and are selected from C_{1-4} alkoxy, aryloxy and arylalkyleneoxy.

81. (New) The method according to claim 80, wherein wherein OR^1 , OR^2 and OR^3 are the same or different and are selected from C_{1-4} alkoxy, phenoxy, naphthyloxy and benzyloxy.

82. (New) The method according to claim 81, wherein wherein OR^1 , OR^2 and OR^3 are the same or different and are selected from C_{1-4} alkoxy.

83. (New) The method according to claim 82, wherein OR^1 , OR^2 and OR^3 are all ethoxy.

84. (New) The method according to claim 39, wherein R^4 is selected from the group consisting of:



wherein n is 0-1.

85. (New) The method according to claim 84, wherein the polyol is an organic di- or polyol.

86. (New) The method according to claim 85, wherein the polyol is selected from the group consisting of a sugar alcohol, sugar acid, saccharide, oligosaccharide and polysaccharide.

Exhibit C

87. (New) The method according to claim 86, wherein the polyol is a selected from the group consisting of allose, altrose, glucose, mannose, gulose, idose, galactose, talose, ribose, arabinose, xylose, lyxose, threose, erythrose, glyceraldehydes, sorbose, fructose, dextrose, levulose, sorbitol, sucrose, maltose, cellobiose, lactose, dextran, (500-50,000 MW), amylose, pectin, glycerol, propylene glycol and trimethylene glycol.

88. (New) The method according to claim 87, wherein the polyol is selected from the group consisting of glycerol, sorbitol, maltose, trehalose, glucose, sucrose, amylose, pectin, lactose, fructose, dextrose and dextran.

89. (New) The method according to claim 88, wherein the polyol is selected from the group consisting of glycerol, sorbitol, glucose, maltose and dextrose.

90. (New) The method according to claim 84 wherein the polymer is a water soluble polymer.

91. (New) The method according to claim 90, wherein the polymer is selected from the group consisting of polyethylene oxide (PEO), polyethylene glycol (PEG), amino-terminated polyethylene oxide (PEO-NH₂), amino-terminated polyethylene glycol (PEG-NH₂), polypropylene glycol (PPG), polypropylene oxide (PPO), polypropylene glycol bis(2-amino-propyl ether) (PPG-NH₂), polyvinyl alcohol, poly(acrylic acid), poly(vinyl pyridine), poly(N-isopropylacrylamide) (polyNIPAM) and polyallylamine (PAM).

92. (New) The method according to claim 91, wherein the water soluble polymer is selected from the group consisting of PEO, PEO-NH₂, PEG, PPG-NH₂, polyNIPAM and PAM.

93. (New) The method according to claim 92, wherein the polymer is PEO.

Exhibit C

94. (New) The method according to claim 84, wherein the linker is selected from the group consisting of C₁₋₂₀alkylene, C₁₋₂₀alkenylene, organic ethers, thioethers, amines, esters, amides, urethanes, carbonates and ureas.

95. (New) The method according to claim 84, wherein the compound of Formula I is selected from one or more of:

GluconamideSi (Compound **1**);

MaltonamideSi (Compound **2**);

DextronamideSi (Compound **3**);

(CH₂CH₂O)_p[(EtO)₃Si(C₃H₆)]₂, p ~4-5, average MW 200 (Compound **5a**);

(CH₂CH₂O)_p[(EtO)₃Si(C₃H₆)]₂, p ~13, average MW 600 (Compound **5b**);

(CH₂CH₂O)_p[(EtO)₃Si(C₃H₆)]₂, p ~44, average MW 2000 (Compound **5c**); and

(CH₂CH₂O)_p[(EtO)₃Si(C₃H₆)]₂, p ~227, average MW 10,000 (Compound **5d**).

96. (New) The method according to claim 1, further comprising combining the organic polyol silane and one or more additives in the presence of one or more biomolecules.

97. (New) The method according to claim 39, further comprising combining the organic polyol silane and one or more additives in the presence of one or more biomolecules.

98. (New) The method according to claim 41, further comprising combining the organic polyol silane and one or more additives in the presence of one or more biomolecules.

100. (New) A method for the quantitative or qualitative detection of a test substance that reacts with, binds to and/or whose reactivity is catalyzed by an active biological substance, wherein said biological substance is encapsulated within a siliceous material, comprising:

Exhibit C

- (a) preparing the siliceous material comprising said active biological substance entrapped within a porous, silica matrix using a method according to claim 98;
- (b) bringing said biological-substance-containing siliceous material into contact with a gas or aqueous solution comprising the test substance; and
- (c) quantitatively or qualitatively detecting, observing or measuring the change in one or more characteristics in the biological substance entrapped within the siliceous material and/or, alternatively, quantitatively or qualitatively detecting, observing or measuring the change in one or more characteristics in the test substance.

101. (New) The method according to claim 100, wherein the change in one or more characteristics of the entrapped biological substance is qualitatively or quantitatively measured by spectroscopy, utilizing one or more techniques selected from UV, IR, visible light, fluorescence, luminescence, absorption, emission, excitation and reflection.

102. (New) A method of storing a biologically active biological substance in a silica matrix, wherein the biological substance is an active protein or active protein fragment, wherein the silica matrix prepared using a method according to claim 98.

EXHIBIT D

Sample 1: DGS (1.00 g, 4.71 mmol) was dissolved in H₂O (1000 μ L) at 0°C by sonication for 20 mins. An aqueous solution of HEPES buffer (1000 μ L) at 50 mM, pH 10, containing 16% PEO (MW=10,000) (w/v) was added and sufficiently mixed. The mixture was allowed to stand at room temperature to gel. Phase separation and gelation occurred after 2 min and 3 min, respectively, to give an opaque hydrogel. The gel was aged at 4 °C overnight, followed by aging at room temperature for 2 days. After washing with H₂O (each time 10 mL, 5 times), and drying in air at room temperature for 1 week, a opaque xerogel was obtained.

Sample 2: PGS was prepared according to the literature (Gill, J. Am. Chem. Soc., (1998), 120, 8587-8598). It was found that PGS was not fully soluble in H₂O. The mixture of PGS (2.00 g) and H₂O (5000 μ L) was sonicated at 0°C for 20 min and filtered (0.62 g of insoluble solid was collected). To the filtrate was added H₂O (300 μ L). 1.60 g of this prehydrolyzed PGS solution contained 0.6 g (4.71) mmol of PGS in 1000 μ L H₂O. Sample 2 was then prepared in a manner similar to sample 1 to provide a semitransparent gel.

Scanning Electron Microscopy (SEM) images for Sample 1 and Sample 2 (see page 2) are shown in Figure 1. A difference in the porosity of the two samples is clearly evident from these pictures. Mercury porosimetry intrusion data (next section) provides quantitative evidence of the differences in pore size between the two samples.

Figure 1: SEM images of DGS-derived (Sample 1) and PGS-derived (Sample 2).

Sample 1 (*DGS/pH 10/8% PEO10K*) (different magnification)

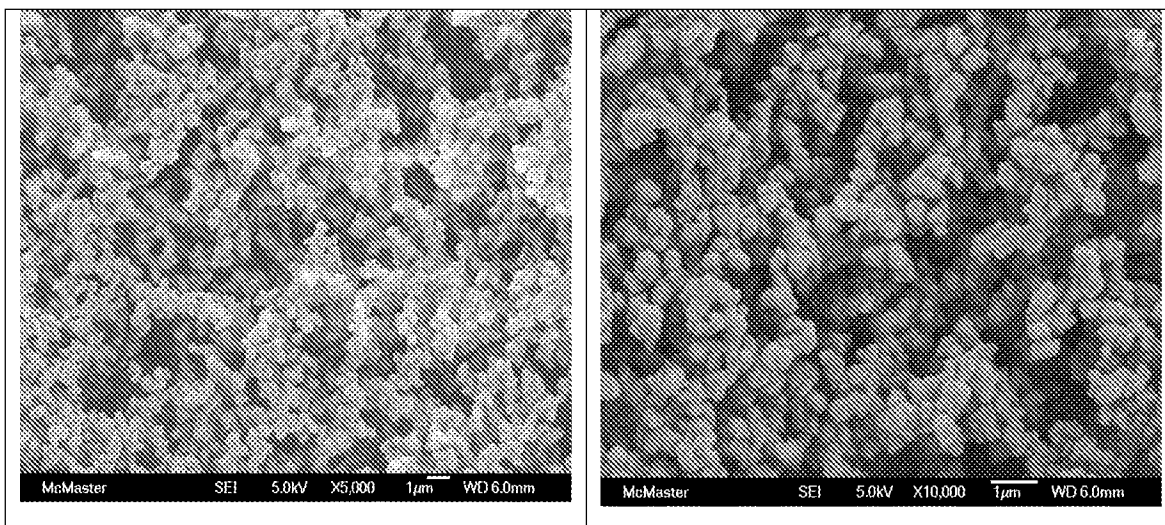


EXHIBIT D

Sample 2 (*PGS/pH 10/8% PEO10K*) (different magnification)

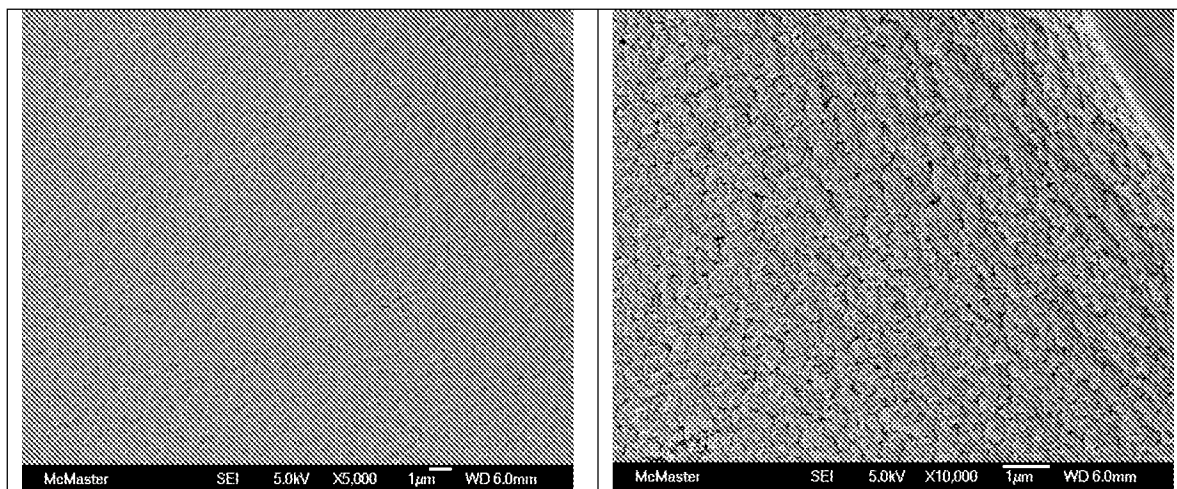


EXHIBIT D

Mercury Porosimetry intrusion Data Summary for DGS-PEO (Sample 1) and PGS-PEO (Sample 2) prepared at pH 10:

The samples were dried at 100°C under vacuum overnight before mercury intrusion porosimetry measurement. Macropore intrusion volumes and macropore size distributions were measured by mercury intrusion porosimetry on a Poremaster GT 60 over a pressure range of 0.10-60,000 psi. The results are shown in Figures 2-4 and in Table 1.

Figure 2. Volume vs pressure curve for DGS-PEO (Sample 1) and PGS-PEO (sample 2) prepared at pH 10.

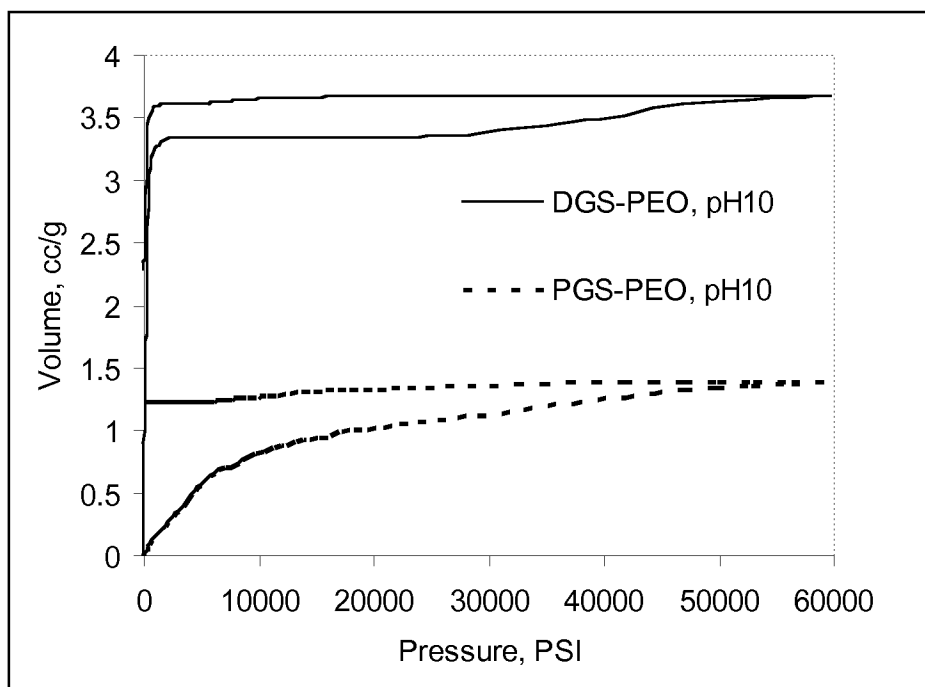


EXHIBIT D

Figure 3. Volume vs pore diameter curve for DGS-PEO (Sample 1) and PGS-PEO (sample 2) prepared at pH 10.

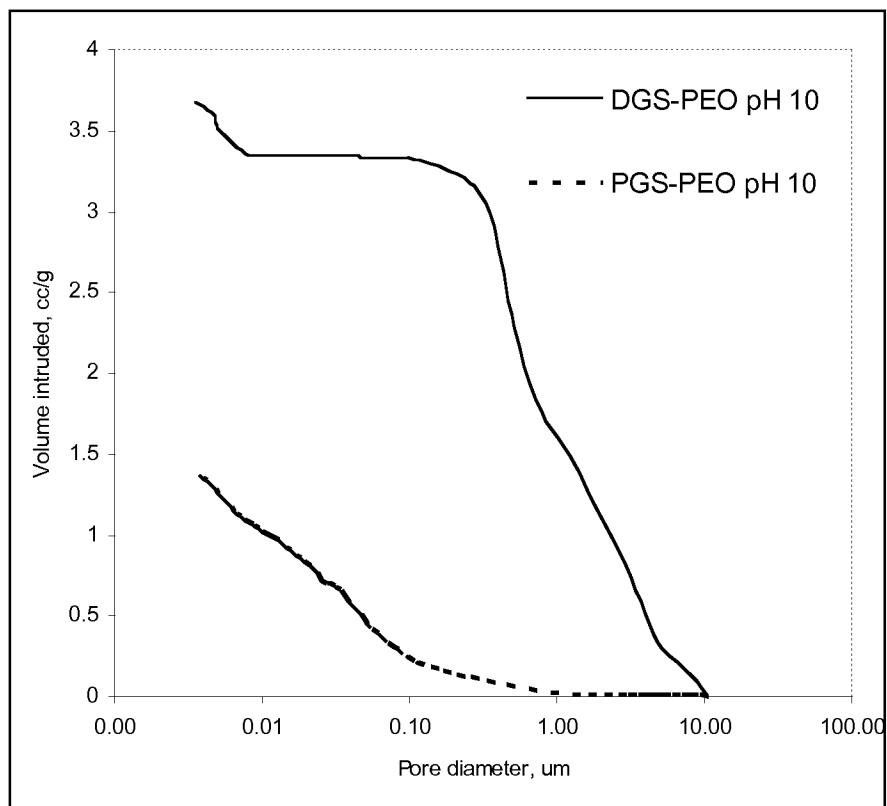


EXHIBIT D

Figure 4. Pore size distribution for DGS-PEO (Sample 1) and PGS-PEO (sample 2) prepared at pH 10.

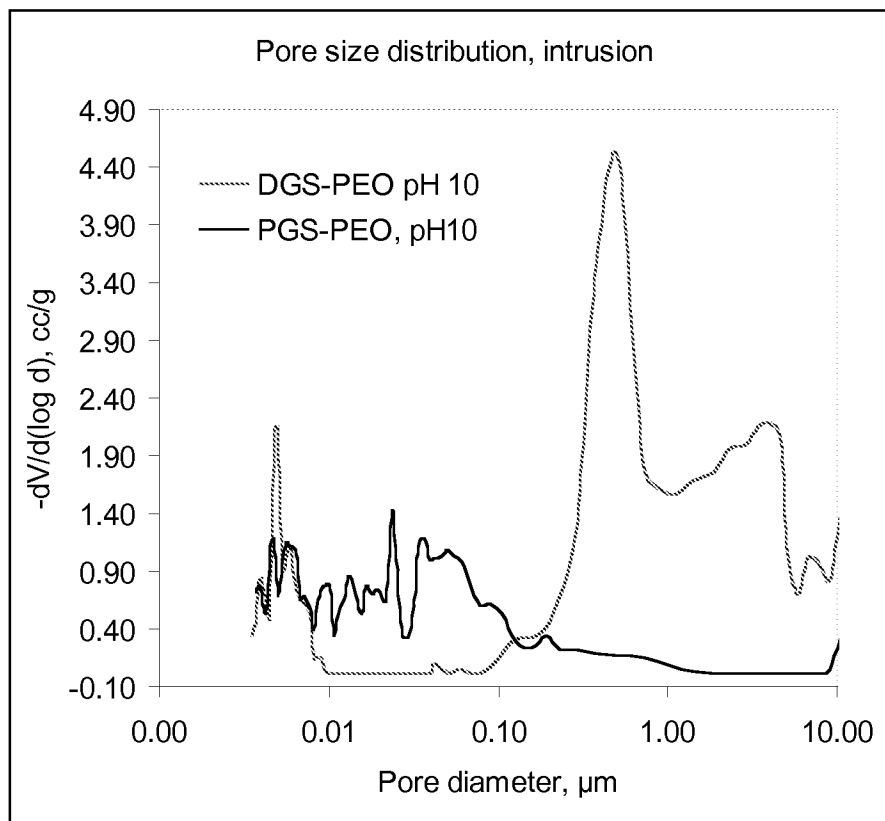


EXHIBIT D

Figure 5. Pore size distribution for DGS-PEO (Sample 1) and PGS-PEO (sample 2) prepared at pH 10

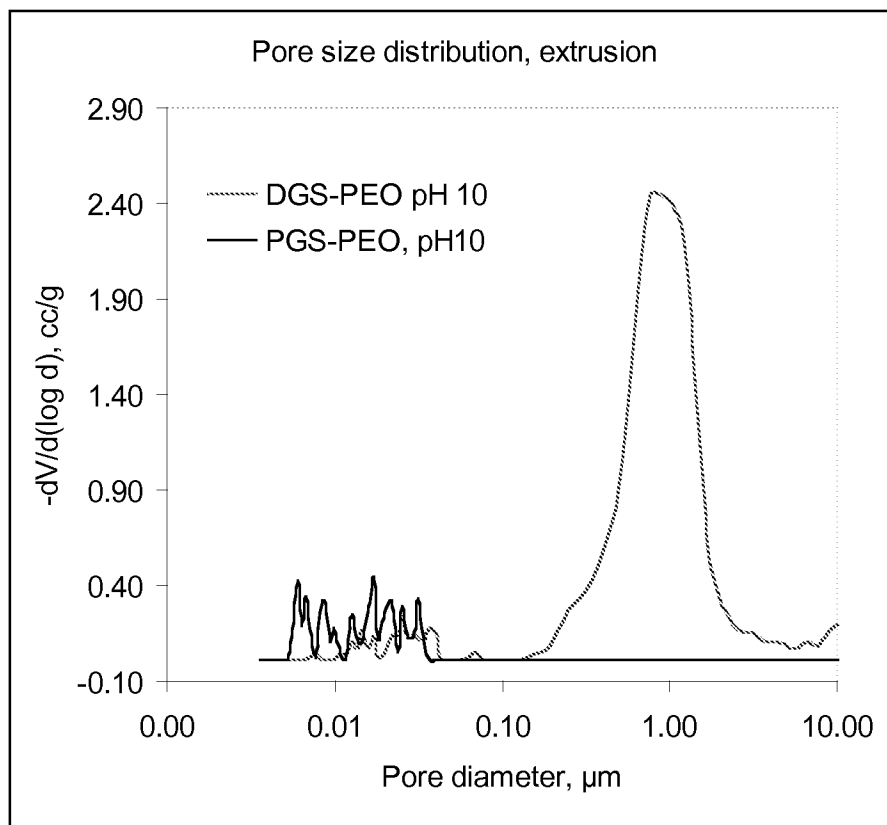


Table 1. Mercury Porosimetry intrusion Data Summary for DGS-PEO (Sample 1) and PGS-PEO (sample 2) prepared at pH 10.

	DGS-PEO	PGS-PEO
Total Surface Area, m^2/g	269.1610	366.2633
Total Intruded Volume, cc/g	3.6647	1.3828
Total Macropore Volume, cc/g	3.3297	0.4544
Total Mesopore Volume, cc/g	0.3350	0.9284
Percentage of Macropore Volume	90.86 %	32.86 %
Percentage of Mesopore Volume	9.14 %	67.14 %
Macropore size (median), μm	0.51 and 4.07	<0.1 (very broad pore size distribution)
Mesopore size(median), μm	0.0048	0.023

EXHIBIT D

Conclusion:

Monolithic silica prepared using the DGS-PEO system at pH 10 exhibits macro/meso bimodal porosity with a total pore volume of 3.6647 cc/g in which the macroporous fraction is up to 90.86%. Macropore size centers on 4.07 and 0.51 μm indicating a broad pore size distribution.

In contrast, the PGS-PEO system at pH 10 only lead to materials with 67.14% mesopores. Mesopore size ranged from 4-50 nm with a broad pore size distribution. Macropore size was less than 100 nm.